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ERRATA

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Cover page 1, January no., for 41, read 39; and for 63 read 61.
Page 117, first line of title, for "infuences" read influences.
Page 355, transpose column headings of columns 4 and 5 in table V. [See page 356, table V—(continued) for correct order.]
Page 431, line 4, for 124, read 724.
Page 643,* omit second sentence of first paragraph.
Page 643, line 6, insert word "single" just before word sodium.
Page 644,* omit sentence beginning in line 21.

* Our paper on automatic conductivity measurements of CO_2 (Plant Physiol. 16: 643–646. 1941) should have included reference to the well-known method of THOMAS and HILL (Plant Physiol. 12: 285–307. 1937). Employing dual solutions, their apparatus automatically records the conductivity of each sodium hydroxide solution at intervals of two minutes. In contrast, our aim was to eliminate the necessity of shifting from one solution to another.—D. G. CLARK, JOHN SHAFER, JR., and O. F. CURTIS.

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A CRITICAL STUDY OF THE NUTRITIONAL REQUIREMENTS OF *PHYMATOTRICHUM OMNIVORUM*[1]

PAUL J. TALLEY AND LESTER M. BLANK

(WITH THREE FIGURES)

Introduction

The causal organism of the root rot disease of cotton and numerous other plants, *Phymatotrichum omnivorum* (Shear) Duggar, has been the subject of numerous studies in the laboratory and in the field (**10**) since it was isolated by ATKINSON (**1**). Some of these laboratory studies have been conducted to test the relative susceptibility of different tissues to the fungus and to test the fungicidal and inhibitory actions of various compounds or decoctions. Many of the culture media employed have been wholly or partly of an unknown chemical nature, although some few synthetic media of known composition and capable of duplication have been employed (**5, 7, 8**). There has been no generally accepted nutrient solution for the culture of this organism. This may be explained by the absence of any comprehensive and critical studies on a synthetic nutrient solution demonstrating that it contains the proper amounts of each of the required components in a combination which results in the most favorable balance for growth. If the composition and characteristics of the nutrient solution are not of a proven satisfactory nature it is obvious that any physiological studies conducted on it are incapable of the most complete evaluation and proper interpretation.

As a basis for a series of studies on the utilization of various materials in the growth of *P. omnivorum* it seemed advisable to undertake a critical study to determine the effects of each required element on the rate and amount of growth, to ascertain the amount of each of these elements that should be present in the nutrient solution, and to determine the influence of one element or compound upon the utilization or effects of the other com-

[1] Published with the approval of the Director as Technical Contribution no. 543 of the Texas Agricultural Experiment Station.

ponents of the solution. The experiments reported herein have been designed to furnish information bearing on these points.

Materials and methods

The culture methods employed in this study are essentially those reported in detail in a previous paper (**2**). A culture of *P. omnivorum*, designated as isolate 28, was grown on potato-dextrose agar in petri dishes and a 5-mm. disc of mycelium and adhering agar was used as the inoculum for each flask. The dry weights of the inoculum pieces were between 3 and 5 mg. in all cases. This isolate, which has been compared in previous studies with several other isolates, may be considered as a representative form of *P. omnivorum*. The composition of the nutrient solutions employed was varied to meet the particular purposes of each experiment. The solutions were not purified by the $CaCO_3$ treatment since this may remove a considerable amount of the phosphates and sulphates and result in solutions whose concentration of the major essential ions is unknown. Reagent grade chemicals were employed and salts furnishing 2 p.p.m. of iron, manganese, and zinc were added in each experiment. These compounds give an adequate supply of the essential minor elements for this organism (**2**). Each experimental solution was prepared so as to give five or more 50-ml. portions, each of which was used in a 250-ml. Erlenmeyer flask as a separate culture. After inoculation the cultures were incubated at 28° C. The individual fungal mats were harvested after time intervals which were determined by the nature of the solutions and purpose of the experiments. In most cases they were harvested when the fungal mass was near or at its maximum weight. This was known to be approximately 21 days after inoculation with this and other rapidly growing isolates when cultured on the more favorable types of media. The dry weights of the individual fungal mats were used to measure the growth response of the organism to the various solutions. Determinations of the pH of the culture solutions were made before inoculation and at the time of harvest.

In an earlier study of the nutritional requirements of *P. omnivorum*, Ezekiel *et al.* (**5**) modified Brown's complete medium (**4**) by using dextrose as the only source of carbon and by supplying the nitrogen as ammonium nitrate. This solution was designated as their solution 70 and has been used as such or with slight modifications, as the basic nutrient solution for several studies on the root-rot organism. In a study of the rôle of the minor or trace elements on the growth of *P. omnivorum* (**2**) this solution with very minor changes was employed as the source of carbon, nitrogen, and other major essential elements. It has proved to be one of the most favorable solutions for the artificial culture of this organism.

In making a critical study of the elemental requirements of *P. omniv-*

orum it was desirable to place the various salts present in this solution on a molar basis, thus facilitating an orderly variation in the amount of each of the salts and their respective ions. This modification of solution 70 has been the basis for the experiments reported herein. The composition of the basic solution used in this study is as follows:

K_2HPO_4	(0.008 M)	1.3940 gm. per liter
$MgSO_4 \cdot 7H_2O$	(0.003 M)	0.7395 gm. per liter
KCl	(0.002 M)	0.1491 gm. per liter
NH_4NO_3	(0.0125 M)	1.0006 gm. per liter
Glucose	(approximately 4%)	40.0000 gm. per liter
Fe, Mn, Zn, each at 2 p.p.m.		

This solution differs from solution 70 by slight decreases in $MgSO_4 \cdot 7H_2O$, KCl, and NH_4NO_3, a slight increase in K_2HPO_4, the omission of $FeCl_3$, and the addition of traces of $FeSO_4$ $7H_2O$, $MnSO_4$ $4H_2O$, and $ZnSO_4$ $7H_2O$.

Experimentation and results

Preliminary experiments demonstrated that the complete omission of any one component of this solution, with the exception of KCl, results in a marked decrease in total growth. With the omission of glucose, NH_4NO_3, $MgSO_4$, or K_2HPO_4 little extension of the mycelium from the inoculum disc is obtained. These results demonstrate the essential nature of certain components but do not prove that each compound, and each ion, is present at the optimum concentration for the maximum rate of growth of *P. omnivorum*. Since the concentration of the minor elements as employed here is at or very near the optimum for the growth of this fungus in an unpurified synthetic medium, any improvement in the composition and balance of the solution, if obtained, would likely be the result of alterations in the concentration of one or more of the major components.

Variation of K_2HPO_4, $MgSO_4$, and KCl

A factorial experiment was designed to test the growth of this fungus when the concentrations of three of the salts were varied. K_2HPO_4 and $MgSO_4$ were studied at three concentrations each while KCl was either present or absent. This was planned and conducted in such a manner that the data could be analyzed statistically by the method of analysis of variance (**3, 6, 9, 11**). The essential principle of this type of experiment is that all the factors investigated, in this case K_2HPO_4, $MgSO_4$, and KCl, are set up in all possible combinations of the concentrations being studied. Information was obtained simultaneously in this way on the response of the organism to each of the different salts and also on the effects of changes in the concentration of each salt on the responses to the others, thus demonstrating the interactions of the several concentrations of these variables.

These interactions are in themselves highly important since they indicate the balance, or lack of balance, between the various salts entering into the nutrient solution. K_2HPO_4 was employed at the basic concentration, 0.008 M, or was decreased to 0.004 M, or increased to 0.012 M. $MgSO_4$ was employed at the basic concentration, 0.003 M, or was decreased to 0.0015 M or increased to 0.006 M. KCl was employed at the basic concentration, 0.002 M, or was omitted entirely. This resulted in 18 different nutrient solutions which were prepared with five replicates of each. The ninety cultures were harvested after 21 days. The weights of the individual mats and pH determinations of each of the five replicates of the 18 treatments are presented in table I. In table II are included the mean values for the reaction of the organism along with the analysis of variance of this experiment.

In table I it is evident that all of these solutions have similar original pH values which are within the desired range for good growth of this organism. The usual increase in acidity as growth progresses is shown by the lower pH values at the time of harvest. This increase in acidity is not as marked in the solutions containing either of the highest two concentrations of K_2HPO_4 as it is in those containing the lowest concentration.

The organism shows considerable variation in its response to the eighteen different solutions. If one assumes that the low mean weights indicate that the solutions on which these mats were produced are inferior to those yielding high mean values, it is possible to evaluate the effect of each of the variants employed in the experiment. The mean values for the reaction of the organism to the various salts or combinations of salts and the analysis of variance (table II) facilitate the interpretation of the rôle of each of the variants in the experiment. The several variables and their effects will be discussed in the order in which they appear in the analysis of variance table. In this paper odds of 99 : 1 are used as the level of statistical significance while odds of 19 : 1 are considered only as indicative.

K_2HPO_4.—There is a significant difference in the response of the organism to the three concentrations of this salt. The mean weight is much lower with the 0.012 M concentration than it is with either 0.004 or 0.008 M. There is no significant difference between the two lowest concentrations.

$MgSO_4$.—The mean weights obtained with the three concentrations of $MgSO_4$ are significantly different. There is a progressive increase in growth as the amount of the salt is increased.

KCl.—The mean weights obtained with and without KCl are not significantly different, there being only a 6-mg. difference in the mean values. This does not prove that the chloride ion is non-essential since slight amounts probably were introduced as impurities in the various components of the solutions. It does show that the organism requires little if any chlorine and that no special addition of chloride is needed to supply it. Potassium can be

TABLE I

GROWTH RESPONSES OF *P. omnivorum* ON COMBINATIONS OF K_2HPO_4, $MgSO_4$ AND KCl AFTER INCUBATING FOR 21 DAYS AT 28° C. FOUR PER CENT. GLUCOSE, 0.0125 M NH_4NO_3, AND 2 P.P.M. EACH OF IRON, MANGANESE, AND ZINC ARE CONSTANT FOR ALL SOLUTIONS

SOL. NO.	SALTS USED, MOLAR CONCENTRATIONS AND ORIGINAL PH OF SOLUTIONS				FINAL PH READINGS: REPLICATES					WEIGHT OF MATS*: REPLICATES					MEAN WEIGHT
	K_2HPO_4	$MgSO_4$	KCl	pH	a	b	c	d	e	a	b	c	d	e	
					pH	*pH*	*pH*	*pH*	*pH*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	0.004	0.0015	0.000	6.74	4.87	4.89	4.89	4.41	5.00	450	535	396	384	501	453
2	0.004	0.0015	0.002	6.78	4.40	4.26	5.06	4.52	4.51	517	508	493	370	565	490
3	0.004	0.0030	0.000	6.81	4.10	4.07	4.19	4.26	4.87	357	534	590	585	568	567
4	0.004	0.0030	0.002	6.84	4.14	3.82	4.43	4.11	4.30	649	527	533	616	625	590
5	0.004	0.0060	0.000	6.77	4.08	3.99	4.08	4.17	4.17	580	621	568	585	553	582
6	0.004	0.0060	0.002	6.78	4.04	4.04	4.05	3.91	4.02	487	548	559	602	593	558
7	0.008	0.0015	0.000	6.92	4.86	4.78	4.67	5.04	5.02	367	346	558	358	490	424
8	0.008	0.0015	0.002	6.93	5.12	6.02	5.90	5.02	5.73	572	508	485	394	355	463
9	0.008	0.0030	0.000	6.83	5.04	4.88	4.70	4.73	4.73	523	567	592	586	616	577
10	0.008	0.0030	0.002	6.82	4.41	4.50	4.85	5.03	4.60	558	555	595	542	548	559
11	0.008	0 0060	0.000	6.72	4.68	4.80	4.90	4.92	4.88	627	643	650	643	498	612
12	0.008	0.0060	0.002	6.72	4.86	5.60	5.05	4.98	5.21	600	496	618	578	578	574
13	0.012	0.0015	0.000	6.93	5.35	5.30	5.28	6.06	5.90	335	341	342	371	414	360
14	0.012	0.0015	0.002	6.92	5.06	5.16	4.88	6.38	5.17	316	451	298	362	317	349
15	0.012	0.0030	0.000	6.88	5.40	6.26	4.71	4.86	4.72	589	363	581	430	477	488
16	0.012	0.0030	0.002	6.88	5.26	4.96	5.72	5.94	4.87	551	523	447	339	527	477
17	0.012	0.0060	0.000	6.85	5.16	5.19	5.03	5.28	5.67	564	621	658	620	475	587
18	0.012	0.0060	0.002	6.85	5.24	5.15	5.12	5.22	5.45	685	633	614	669	621	645

* Fractional milligrams are not recorded in this table, although they were taken into consideration in the subsequent analyses.

TABLE II

MEAN VALUES AND ANALYSIS OF VARIANCE FOR THE GROWTH RESPONSES OF *P. omnivorum* ON COMBINATIONS OF K_2HPO_4, $MgSO_4$, AND KCl

MEAN WEIGHTS IN MG. FOR

K_2HPO_4 Concentration	Mean
0.004	540
0.008	535
0.012	484

$MgSO_4$ Concentration	Mean
0.0015	423
0.0030	543
0.0060	593

KCl Concentration	Mean
0.000	517
0.002	523

$K_2HPO_4 \times KCl$ Concentration	Mean
0.004 × 0.000	534
0.002	546
0.008 × 0.000	538
0.002	532
0.012 × 0.000	479
0.002	490

$MgSO_4 \times KCl$ Concentration	Mean
0.0015 × 0.000	413
0.002	434
0.0030 × 0.000	544
0.002	542
0.0060 × 0.000	594
0.002	592

$K_2HPO_4 \times MgSO_4$ Concentration	Mean
0.004 × 0.0015	472
0.0030	578
0.0060	570
0.008 × 0.0015	443
0.0030	568
0.0060	593
0.012 × 0.0015	355
0.0030	483
0.0060	616

$K_2HPO_4 \times MgSO_4 \times KCl$ Concentration	Mean
0.004 × 0.0015 × 0.000	453
0.002	490
0.0030 × 0.000	567
0.002	590
0.0060 × 0.000	582
0.002	558
0.008 × 0.0015 × 0.000	424
0.002	463
0.0030 × 0.000	577
0.002	559
0.0060 × 0.000	612
0.002	574
0.012 × 0.0015 × 0.000	360
0.002	349
0.0030 × 0.000	488
0.002	477
0.0060 × 0.000	587
0.002	645

ANALYSIS OF VARIANCE

Variance	D.F.	Mean square	F Found	F Required odds 99 : 1	F Required odds 19 : 1
Total	89	10,054.0245			
K_2HPO_4	2	28,257.3854	7.42	4.92	3.13
$MgSO_4$	2	228,328.5004	59.95		
KCl	1	826.8871			
$K_2HPO_4 \times MgSO_4$	4	21,040.5447	5.52	3.60	2.50
$K_2HPO_4 \times KCl$	2	759.0325			
$MgSO_4 \times KCl$	2	1,329.0488			
$K_2HPO_4 \times MgSO_4 \times KCl$	4	4,561.5801	1.20		
Error	72	3,808.6787			

Required "F" and "t" values taken from SNEDECOR's table (9).
Difference required for significance between means for odds of 99 : 1 for
K_2HPO_4 or $MgSO_4$ = 42.19 mg.
KCl = 34.00 mg.

furnished in adequate quantities in other compounds and the small additional amount of potassium furnished by KCl in this experiment had no consistent effect.

INTERACTIONS OF THE VARIOUS SALTS.—Interaction of the variables in this experiment are important since they give a direct measurement and evaluation of the physiological relationships of the various salts used in these solutions. As an aid in visualizing these interactions the results are presented

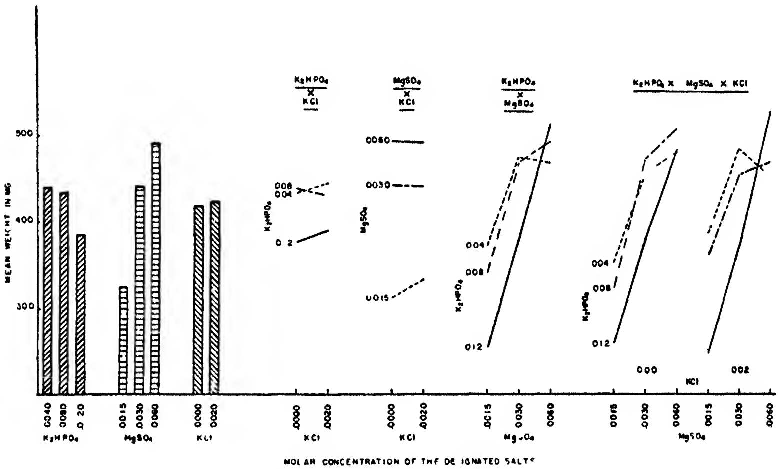

FIG. 1. The mean responses of *P. omnivorum* to different concentrations of K_2HPO_4, $MgSO_4$, and KCl and curves representing the first and second order interactions of these salts on the growth of the fungus.

graphically in figure 1. The three groups of vertical bars indicate the main effects of the several concentrations of each of the three salts. To the right of the vertical bars are graphs of the three first order interactions, $K_2HPO_4 \times MgSO_4$, $K_2HPO_4 \times KCl$, and $MgSO_4 \times KCl$. At the extreme right are shown the mean values of the second order interaction, $K_2HPO_4 \times MgSO_4 \times KCl$.

INTERACTION OF $K_2HPO_4 \times MgSO_4$.—This interaction is significant. In the table of mean values for this interaction (table II) the benefits of increased concentrations of $MgSO_4$ are shown to be more pronounced as the concentration of K_2HPO_4 is increased. The proper physiological balance between these two salts is just as important as the direct effects of either one. If the proper balance does not exist, the advantages of low K_2HPO_4 and of high $MgSO_4$ do not apply. This is shown very clearly in the graph (fig. 1) for the interaction of $K_2HPO_4 \times MgSO_4$. The combinations of 0.004 and 0.008 M K_2HPO_4 with 0.003 M $MgSO_4$ are equally good. By increasing the concentration of the latter salt to 0.006 M the solutions with 0.004 M K_2HPO_4 are impaired slightly while those with 0.008 M K_2HPO_4 are improved. The importance of this balance is more noticeable with the highest concentration

of K_2HPO_4 (0.012 M) where good growth is obtained only in combination with the highest concentration of $MgSO_4$ (0.006 M).

The remaining interactions, $K_2HPO_4 \times KCl$, $MgSO_4 \times KCl$, and $K_2HPO_4 \times MgSO_4 \times KCl$, are not significant as shown by the analysis of variance (table II).

Summarizing the main effects of the three salts as shown in this experiment, it is evident that the higher mean weights are obtained with the lower concentrations of K_2HPO_4 and with the higher concentrations of $MgSO_4$, while the addition or omission of KCl is of no consequence. It is further evident that these main effects of the individual salts cannot be extended indefinitely, since a balance between K_2HPO_4 and $MgSO_4$ is extremely important.

Variation of K_2HPO_4, $MgSO_4$, and NH_4NO_3

A second experiment was devised on the factorial design in which NH_4NO_3, K_2HPO_4, and $MgSO_4$ were varied at three concentrations each while KCl was omitted entirely from the nutrient solutions. Preliminary studies have indicated that benefits could be obtained by increasing the concentration of NH_4NO_3. In this second experiment NH_4NO_3 was used at 0.0125, 0.025, and 0.0375 M concentrations. The results of the preceding experiment suggested that K_2HPO_4 be studied at the 0.0048, 0.0064, and 0.008 M concentrations and that $MgSO_4$ be supplied at the 0.0045, 0 006, and 0.0075 M concentrations. Four replicates of each of the twenty-seven different solutions were harvested after twenty-one days, which was the period of incubation used in the preceding factorial experiment. The original pH values of the solutions fall between 6.64 and 6.90. Since replicates within a treatment are extremely uniform the individual weights are omitted. The mean values and the analysis of variance are presented in table III. The results are presented graphically in figure 2. The variants and their interactions will be discussed in the order in which they appear in table III.

NH_4NO_3.—Significant differences exist between all three concentrations of NH_4NO_3. The mean weights increase as the concentration of this salt increases. The 0.025 and 0.0375 M concentrations yield mean weights which are approximately twenty-five per cent. greater than the mean weights for 0.0125 M concentration.

K_2HPO_4.—A significant difference is attained between the mean weights for the three concentrations of this salt. The best growth occurs with the highest concentration of K_2HPO_4. The variation in the responses to the three concentrations is not as great as that with the three concentrations of NH_4NO_3.

$MgSO_4$.—The differences between the mean weights for the several concentrations of $MgSO_4$ are not great enough to be significant at odds of 99 : 1 but are significant at odds of 19 : 1. The heaviest mats are produced by

TABLE III

MEAN VALUES FOR THE REACTION OF *P. omnivorum* TO COMBINATIONS OF NH_4NO_3, K_2HPO_4, AND $MgSO_4$

MEAN WEIGHTS IN MG. FOR

NH_4NO_3

CONCENTRATION	MEAN
0.0125	593
0.0250	758
0.0375	772

K_2HPO_4

CONCENTRATION	MEAN
0.0048	686
0.0064	705
0.0080	732

$MgSO_4$

CONCENTRATION	MEAN
0.0045	712
0.0060	713
0.0075	698

$K_2HPO_4 \times MgSO_4$

CONCENTRATION	MEAN
0.0048 × 0.0045	699
0.0060	693
0.0075	666
0.0064 × 0.0045	710
0.0060	700
0.0075	705
0.0080 × 0.0045	727
0.0060	745
0.0075	724

$NH_4NO_3 \times K_2HPO_4$

CONCENTRATION	MEAN
0.0125 × 0.0048	561
0.0060	602
0.0075	616
0.0250 × 0.0048	731
0.0060	752
0.0075	790
0.0375 × 0.0048	766
0.0060	761
0.0075	789

$NH_4NO_3 \times MgSO_4$

CONCENTRATION	MEAN
0.0125 × 0.0045	597
0.0060	593
0.0075	589
0.0250 × 0.0045	762
0.0060	754
0.0075	758
0.0375 × 0.0045	777
0.0060	791
0.0075	750

$NH_4NO_3 \times K_2HPO_4 \times MgSO_4$

CONCENTRATION	MEAN
0.0125 × 0.0048 × 0.0045	564
0.0060	563
0.0075	555
0.0064 × 0.0045	604
0.0060	604
0.0075	598
0.0080 × 0.0045	622
0.0060	612
0.0075	615
0.0250 × 0.0048 × 0.0045	748
0.0060	717
0.0075	728
0.0064 × 0.0045	756
0.0060	749
0.0075	752
0.0080 × 0.0045	781
0.0060	795
0.0075	794
0.0375 × 0.0048 × 0.0045	784
0.0060	798
0.0075	716
0.0064 × 0.0045	771
0.0060	748
0.0075	764
0.0080 × 0.0045	777
0.0060	827
0.0075	764

ANALYSIS OF VARIANCE

VARIANCE	D.F.	MEAN SQUARE	F FOUND	F REQUIRED ODDS 99:1	F REQUIRED ODDS 19:1
Total	107	7,836.70			
NH_4NO_3	2	356,130.27	565.24	4.88	3.11
K_2HPO_4	2	19,106.06	30.32		
$MgSO_4$	2	2,285.10	3.63		
$NH_4NO_3 \times K_2HPO_4$	4	2,129.83	3.38	3.56	2.49
$NH_4NO_3 \times MgSO_4$	4	1,905.97	3.03		
$K_2HPO_4 \times MgSO_4$	4	1,543.09	2.45		
$NH_4NO_3 \times K_2HPO_4 \times MgSO_4$	8	1,266.77	2.01	2.74	2.06
Error	81	630.05			

Difference required for significance between means for odds 99:1 for NH_4NO_3, K_2HPO_4, or $MgSO_4$ = 15.61 mg

solutions containing $MgSO_4$ at 0.0045 and 0.006 M concentrations. Nothing is gained by increasing the concentration to 0.0075 M.

INTERACTIONS.—In a further analysis, the multiple degrees of freedom for the main contributors and the several interactions shown in table II were broken down. No additional information was gained with the exception that one of the eight comparisons for the second order interaction reaches significance at odds of 99 : 1. An inspection of figure 2 suggests that this might have been anticipated. There it will be noted that the different concentrations of $MgSO_4$ are quite without effect at all concentrations of K_2HPO_4 when NH_4NO_3 is at 0.0125, but when the latter is at 0.0375 the intermediate concentration of $MgSO_4$ is superior and the high concentration is decidedly harmful at both the low and high rates for K_2HPO_4. While one of the comparisons in the second order interaction reaches significance at odds of 99 : 1, none of the first order interactions does, although certain of them reach the 19 : 1 requirement. These relationships are shown quite clearly in figure 2, and no further elaboration is deemed necessary.

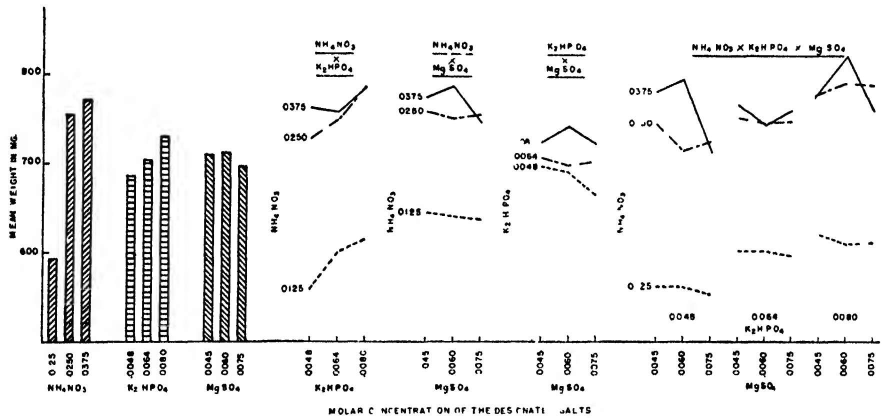

FIG. 2. The mean responses of *P. omnivorum* to different concentrations of NH_4NO_3, K_2HPO_4, and $MgSO_4$ and curves representing the first and second order interactions of these salts on the growth of the fungus.

With the exception of the additional information resulting from the use of the three concentrations of NH_4NO_3 and the interactions of this salt with K_2HPO_4 and $MgSO_4$ these results are essentially what one would expect on the basis of the first factorial experiment. The three concentrations of K_2HPO_4 cover a relatively narrow range and fall between the lowest two concentrations used in the earlier experiment, while the three concentrations of $MgSO_4$ vary around the highest concentration used in the preceding experiment. Under these conditions $MgSO_4$ loses significance since all three concentrations are equal to, or greater than, the amount required for the best

growth. The three concentrations of K_2HPO_4, however, become significant since all are below the optimum amount required to maintain the balance indicated in the first factorial experiment. On this basis it is logical to expect that each of these slight increases in the concentration of K_2HPO_4 would produce significantly more growth since all solutions contain $MgSO_4$ at relatively high concentrations.

The effects of the three concentrations of NH_4NO_3 used in this experiment are outstanding (fig. 2). A very large increase in growth results when the nitrogen content is doubled. When nitrogen is increased beyond this point the relative gain in growth diminishes. The highest concentration of NH_4NO_3 tends to modify the influence of the other salts present in the solutions. The significant interaction, at odds of 19 : 1, of $NH_4NO_3 \times K_2HPO_4$ is a result of the relatively minor differences in the response to the different levels of K_2HPO_4 when NH_4NO_3 is at the 0.0375 M concentration. In a like manner this highest concentration of NH_4NO_3 has a reaction with the three concentrations of $MgSO_4$ different from the lowest two concentrations of NH_4NO_3. Since the additional gain in growth is slight with the last increase in NH_4NO_3 and the reactions to K_2HPO_4 and $MgSO_4$ are of a variable nature with this highest concentration, it is doubtful that the concentration of NH_4NO_3 should be increased above 0.025 M.

Importance of balanced solution

The direct effects of K_2HPO_4 and $MgSO_4$ and the desirable balance between them suggested the advisability of testing the changes in the amount of growth that might be produced if these two salts were varied in a manner which would maintain the same balance in all solutions but would vary the amounts of each of the salts simultaneously. Such an experiment was designed on the factorial basis. In this experiment the balance of the various components was the same throughout, the ratio for the salts being the same as in a solution containing 0.008 M K_2HPO_4, 0.006 M $MgSO_4$, and 0.002 M KCl. The solutions were varied by reducing the concentration of these respective salts one half in one series of solutions, and by increasing the concentration twice in another and four times in still another. This gave four treatments having the same balance but the ratio of the concentration of these salts was 0.5 : 1 : 2 : 4. Each of these four solutions was tested with 0.0125 and 0.025 M NH_4NO_3, while glucose and the trace elements were constant in all solutions. Harvests were made at 18 and 24 days.

There is no significance to the slightly different mean values for the four different concentrations of the salts. As in the preceding experiment a significant difference exists between the two concentrations of NH_4NO_3. The interaction of NH_4NO_3 with the four concentrations of salts lacks significance.

This factorial experiment proves that the concentrations of any one salt can be varied over a wide range if the salts supplying the other major essential elements are varied in a like manner, thus emphasizing the importance of the balance between K_2HPO_4 and $MgSO_4$. It also indicates that the proportion of the various salts are near the optimum balance for the best growth under the conditions employed in these studies.

Variation of single ions

The experiments in which the several salts are varied individually and in combinations show that little, if any, further improvement in the nutritional properties of the basic solution can be obtained by this method. Two or more ions are varied simultaneously, however, in every modification obtained by varying the concentration of any of the individual salts. It seemed possible that the fungus might give a better growth response to a change in the concentration of one ion of a salt which would be offset by a less favorable response to a corresponding change in the other ion or ions contributed by this same salt.

An experiment was designed in which each separate solution differs from the control by a variation in the concentration of only one major essential ion. The constant factors throughout are 0.025 M NH_4NO_3, 4 per cent. glucose, and 2 p.p.m. of iron, manganese, and zinc. The composition of the control, which is given in table IV as solution 11, is 0.008 M K_2HPO_4, 0.006 M $MgSO_4$, and 0.001 M KCl. Solutions 1 to 10 differ from the control by having the molar concentration of one major ion increased, or decreased, by one-half the amount in the control. The nature of this experiment necessitates the use of several different compounds as sources of the essential ions. In each solution only one ion is varied with the single exception of the solution with low phosphate (solution 3) in which the chloride ion is increased slightly. An additional solution was used to show the possible effect of this second change. No significant effect results from this increase in the concentration of chlorine. The basic solution which served as a starting point for these studies is included (solution 12) for comparison with the control (solution 11). Sufficient replicates of each of the solutions were prepared to permit the harvest of four cultures of each at 18 and at 24 days. The derivation and composition of the various solutions, their original pH values, and the ranges of pH at times of harvest are given along with the mean weights of the mats in table IV.

Analyses of variance were made for each of the groups of ions for the first harvest, thus comparing the effects of the low, high, and intermediate concentrations of each ion during this period. The differences between concentrations are of no significance with the exception of the concentrations of the potassium ion (solutions 1, 2, and 11). The significance in this

TABLE IV

Growth responses of *P. omnivorum* to variation in the concentrations of the K, PO_4, Mg, SO_4, and Cl ions varied singly. Four per cent. glucose, 0.025 M NH_4NO_3, and 2 p.p.m. each of iron, manganese, and zinc are constant for all solutions

Sol. no.	Molar concentration of variable ion	Salts used, molar concentrations and original pH of solutions								Growth response at harvest			
										18 days		24 days	
		K_3PO_4	K_2HPO_4	KH_2PO_4	$Mg_3(PO_4)_2$	$MgSO_4 \cdot 7H_2O$	K_2SO_4	KCl	pH	pH range of solutions	Mean weight of mats	pH range of solutions	Change in mat weight, 18 to 24 days
										pH	*mg.*	*pH*	*mg.*
1	0.009 K			0.008		0.006		0.001	4.30	3.24–3.30	63.1	3.20–3.28	− 9.5
2	0.025 K	0.008				0.006		0.001	7.33	6.49–6.70	707.3	5.50–6.38	− 26.7
3	0.004 PO_4	0.004				0.006		0.005	7.20	4.72–5.67	744.0	5.28–6.09	− 89.3
4	0.012 PO_4		0.004	0.008		0.006		0.001	6.07	4.54–5.61	782.7	5.05–5.45	− 86.1
5	0.003 Mg		0.002	0.006		0.003	0.003	0.001	5.80	3.88–4.24	760.4	4.51–4.90	−112.5
6	0.009 Mg	0.005		0.001	0.001	0.006		0.001	7.21	6.32–6.60	730.3	5.37–5.72	+ 4.0
7	0.003 SO_4	0.005		0.001	0.001	0.003		0.001	7.27	6.47–6.70	742.3	5.43–6.22	− 22.4
8	0.009 SO_4		0.002	0.006		0.006	0.003	0.001	5.87	3.60–4.40	763.7	4.92–5.20	− 54.6
9	0.000 Cl	0.001	0.007			0.006			6.76	5.41–6.30	779.6	5.18–5.94	− 82.7
10	0.002 Cl			0.001		0.006		0.002	6.53	4.80–5.94	780.2	5.67–6.05	− 82.5
11	0.017 K 0.008 PO_4 0.006 Mg 0.006 SO_4 0.001 Cl		0.008			0.006		0.001	6.62	5.21–5.93	767.9	5.40–6.13	− 53.0
12	0.018 K 0.008 PO_4 0.003 Mg 0.003 SO_4 0.002 Cl		0.008			0.003		0.002	6.59	4.79–5.22	788.2	4.78–5.62	− 82.0

comparison is the result of the almost complete suppression of growth in solution 1 which contains the lowest concentration of potassium. A comparison of solutions 11 and 12 fails to show a significant difference.

Similar analyses of variance were made for the second harvest (24 days). The mean weights for the high, low, and intermediate concentrations of each ion are more variable than they were at 18 days. Significant differences exist between the various concentrations of the phosphate and magnesium ions as well as the potassium ion. These two new significant differences can be discounted since they occur only after autolysis has set in. This is indicated by the rise in pH and the decrease in the mean weights for these particular solutions in the interval between the first and second harvests. The only solution that does not produce heavier mats in 18 days, in contrast to 24 days, is the one containing high magnesium. There is apparently a decreased rate of growth with this combination (solution 6) so that the peak is reached at a later date. This experiment demonstrates the adequacy of, and balance between, each of the ions supplied in the control solution. The ranges of the balances between the different ions are either very broad or else poorly defined, with the exception of those involving potassium.

The effect of the potassium ion in this experiment may be attributed to either one or both of two different factors. The earlier experiments show the importance of a balance between K_2HPO_4 and $MgSO_4$. It could be assumed that the low potassium content of solution 1 produces a poor balance between potassium and either one or both ions of $MgSO_4$, since the actual ratio of potassium to magnesium or sulphate in this solution is almost the reverse of that present in the better salt combinations obtained in the factorial experiments. If this assumption is true the main effect of K_2HPO_4 in the balance between K_2HPO_4 and $MgSO_4$ might be attributed to potassium alone, since solution 3 containing a decreased amount of the phosphate ion is not out of balance. Another factor entering into any explanation of the poor growth in solution 1 is the high concentration of hydrogen ions and the still further increase in the acidity of the solution during the period of incubation. *P. omnivorum* does not grow well in an acid solution (5). It is probable that the low pH values for this solution account for most of the poor response on the part of the organism.

No two of the twelve solutions listed in table IV have the same composition but eleven of them are satisfactory for the growth of this organism. The source of the essential elements is unimportant. A good nutrient solution can be prepared by using the proper combinations of any of the phosphates and sulphates of potassium and magnesium as the source of potassium, magnesium, phosphorus, and sulphur, if the concentration of the individual ions in the resulting solution meet the requirements for the proper supply and balance for growth. There are numerous solutions

which will serve as equally satisfactory sources of the major essential elements for the growth of *P. omnivorum*. One of the best of these is the original solution containing 0.008 M K_2HPO_4, 0.003 M $MgSO_4$, and 0.002 M KCl with the concentration of NH_4NO_3 increased to 0.025 M. It cannot be improved significantly by increasing or decreasing the amount of any of these major inorganic components.

Variation of glucose and NH_4NO_3

The responses of the organism to variations in concentration of the inorganic salts have been studied thus far with the carbon source held constant at four per cent. glucose. An experiment was designed in which glucose is supplied at one, two, four, or eight per cent. and NH_4NO_3 is supplied at

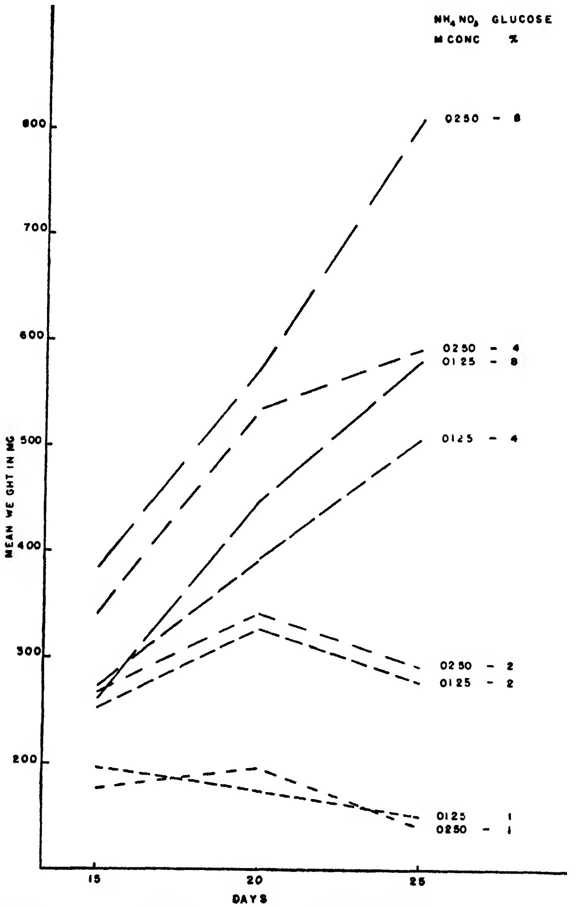

FIG. 3. The influence of different amounts of NH_4NO_3 and glucose on the growth of *P. omnivorum*.

0.0125 or 0.025 M concentrations. The constant factors in the solution are 0.008 M K_2HPO_4, 0.003 M $MgSO_4$, 0.002 M KCl and iron, manganese, and zinc at 2 p.p.m. Sufficient replicates of each of the eight resulting solutions were employed to permit the harvest of four cultures of each at several time intervals. The experiment was repeated several times, varying the periods of incubation. The results are presented graphically in figure 3, and are based on the combined values obtained at 15, 20, and 25 days in each of three different experiments.

The results can be interpreted in terms of total growth, rate of growth, or efficiency of the utilization of glucose. The curves in figure 3 indicate that the total amount of growth is determined more by the amount of glucose in these solutions than by the amount of nitrogen. If sugar is limiting there is little to be gained by increasing the nitrogen content of the solution. The rate of growth when sugar is not limiting is influenced by the concentration of NH_4NO_3. This regulatory action of NH_4NO_3 becomes more noticeable as the sugar content is increased. As the peak of growth is approached, or attained, there is little choice between the two nitrogen levels unless the sugar content is extremely high (8 per cent.). Only with this high content of sugar does the 0.0125 M concentration of NH_4NO_3 become limiting. The only constant effect of NH_4NO_3 in solutions containing 4 per cent. sugar is the increased rate of growth.

More growth can be obtained by increasing the sugar content of the solution with either concentration of NH_4NO_3 but the apparent efficiency of carbon utilization decreases. The concentration of NH_4NO_3 regulates the rate of carbon utilization but does not materially alter the apparent efficiency of this utilization unless the sugar content is above 4 per cent.

Summary and conclusions

The nutritional requirements of *P. omnivorum* in synthetic solutions were studied. Factorial experiments were employed to obtain data on the direct effect of each of the major components in the solution and on the interactions of certain of these compounds.

The proper balance between dibasic potassium phosphate and magnesium sulphate is as important as the direct effect of either salt. If the concentration of one of these salts is increased appreciably the concentration of the other salt should be increased accordingly. If the proper balance is maintained the concentrations of dibasic potassium phosphate and magnesium sulphate can be decreased fifty per cent. or increased four hundred per cent., with no significant change in the amount of growth obtained. The potassium ion is more important in this balance than the phosphate radical.

The chloride ion is either non-essential or is furnished in sufficient quantities as impurities in the reagents.

There is no significantly superior combination of salts for the supply of the inorganic nutrients. The range of tolerance for each of the major essential ions is relatively broad.

A solution containing 0.008 M dibasic potassium phosphate, 0.003 M magnesium sulphate, 0.002 M potassium chloride, and 2 p.p.m. of iron, manganese, and zinc is not significantly improved by increasing or decreasing the concentration of any one of these salts or their ions. This solution is well balanced and the amount of growth developing on it is determined by the supply of nitrogen or the available carbon.

The rate of growth increases as the supply of ammonium nitrate increases. This holds true only over a certain range beyond which the growth increment decreases rapidly. With extremely high concentrations of ammonium nitrate the responses to the different concentrations of dibasic potassium phosphate and magnesium sulphate are irregular. The increased growth rate obtained with higher concentrations of nitrogen does not increase the efficiency of carbon utilization.

If the carbon source, glucose, is not a limiting factor the rate of growth may be regulated by the nitrogen supply. If glucose is limiting there is little if any gain obtained by increasing the nitrogen supply.

Increasing the carbon supply increases the amount of growth but lowers the apparent efficiency of carbon utilization.

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COMPARISON OF RATES OF WATER INTAKE IN CONTIGUOUS REGIONS OF INTACT AND ISOLATED ROOTS

HILDA F. ROSENE

(WITH SEVEN FIGURES)

Introduction

Although extensive investigations dealing with the problem of water absorption in roots have been carried out for many years, practically no attempt has been made to obtain quantitative data on water absorption by root tissue *per se*. Most of the investigations in the past have been made on intact and topped plants. When, however, it is desirable to compare the activities of the root in the presence of shoot influences with those in its absence, it is imperative that all of the shoot tissue be removed; since it has been shown (**16**) that auxin applied to the topped stem influences translocation of water and solutes in the roots, it may be that substances produced by the stem will do the same.

It might be objected that roots removed from their "normal" connections by excision are in an "abnormal" state. Such roots nevertheless continue to grow and transport water and solutes for many hours after excision. As pointed out later, a given region in a single root under controlled conditions may even transport greater volumes of water *after* excision.

Experimental studies on isolated roots and pieces of roots under properly controlled conditions may yield results that are of fundamental significance to a basic understanding of the dynamics of water transport in all tissues. Indeed, many of the basic problems of solute absorption and accumulation have been effectively studied on excised roots by HOAGLAND (**4**), HOAGLAND and BROYER (**5**), PREVOT and STEWARD (**11**), and STEWARD (**17**). With respect to water transport in root tissue *per se*, WHITE (**18**) demonstrated that there was a continuous unidirectional flow through isolated tomato roots growing *in vitro* and that such roots manifest six atmospheres or more of root pressure.

Investigations on the direct determination of water absorption by various root regions are few in number. These have not been carried out under conditions or by techniques which make the results strictly comparable. Even the objectives of the investigators have been different. To obtain consistent results such variables as age and nutritional history of the roots, seasonal effects on the development of the root systems, and variability of material must be taken into consideration (HOAGLAND and BROYER, **5**). ROSENE (**13**) demonstrated that the magnitude and distribution of rates of

water absorption in a single root change with aging of the tissues. Unless authors state the age of roots as well as the length, comparisons are not as significant as they might otherwise be. The structural differentiation of a 65-mm. onion root six days old, for example, is very different from that of a 65-mm. root which has grown slowly and is three weeks old; the first has no lateral branches, the second may be profusely branched. In the investigations of Höhn (6), Sierp and Brewig (15), and Gregory and Woodford (3) water intake by different root regions was expressed in centimeter length of root. Rosene (13) by means of a different technique determined simultaneously unit flow in unit time through unit surface area in the different root regions. Because of differences in diameter of a single root from apex to base and of different roots, significance of comparisons of the results of the different investigators mentioned above are limited.

Höhn (6), working with roots of *Zea mays* and *Tradescantia fluminensis*, Sierp and Brewig (15) with roots of *Vicia faba*, *Zea mays*, and *Ricinis communis*, and Rosene (13) with roots of *Allium cepa* conclude that the entire surface of roots less than 10 cm. in length is capable of water absorption. Both Höhn and Rosene maintain that relatively little absorption occurs in the apical meristem, while Sierp and Brewig conclude that this region frequently manifests water loss. Höhn, Sierp and Brewig, and Rosene are in agreement that maximum rates of water absorption are exhibited by relatively more basal regions in roots less than 70 mm. in length.

Up to the present no study had been made to determine the rates of water absorption in different regions of an individual root both before and after excision. The present investigation was carried out with this purpose in mind. It serves as a foundation and departure for further studies of water transport by root tissue.

Experimentation

Method

The investigation was restricted to relatively young onion roots (*Allium cepa*) less than 65 mm. in length and not more than a week old. The onions were readily cultured in aerated solutions—Trelease culture solution or in nutrient tap water.[1] Ordinarily the roots develop no laterals in culture solutions until they are over 2 weeks old. Since the present study did not include effects of transpiration on water intake and since relatively long roots often developed before leaves, the roots were cultured in the dark. This appeared feasible because the bulb furnished a ready supply of carbohydrate. It also simplified experimental technique since the entire plant

[1] Analyses of tap water were kindly furnished at intervals by Prof. E. P. Schoch, Director of the Bureau of Industrial Chemistry.

could be transferred to a single experimental chamber in which it was not necessary to illuminate the leaves at the same time that the roots were protected from light. No analyses of roots were made; they were of unknown sugar and salt content.

Detailed description of the apparatus and technique employed are omitted since these have been published (**13**). It is, therefore, necessary to describe only those modifications in procedure which are pertinent to the present investigation. Having placed the plant in the moist chamber, the cover was not removed until the experiment was complete. This was possible because all manipulations were precisely controlled from the outside. When desired, fluid could be added to or withdrawn from the potometers, menisci adjusted, the root excised or cut into segments, and the potometers or the root raised and lowered without disturbing the interior atmosphere. Rates of elongation, the average diameter of the root, height of the water column at each root contact, and the movement of the terminal meniscus in each potometer were measured with an eyepiece micrometer. No water flow along the root from one potometer to that below was observed. Several methods, including the use of dyes, were used to check on this possibility. Probably the most convincing check that higher rates were observed at higher levels because of inherent characteristics of the root itself and not fortuitous circumstances was the fact that when the root was inverted the higher rates of basal levels produced greater withdrawal of water from the lower potometers. Clear cut water columns without merging of menisci were maintained when potometers were placed side by side and the distance between the menisci of the separate root contacts was 0.2 mm. There was no difficulty with the appearance of bubbles at either the potometer contact or within the potometer tube.

A duplicate potometer tube, separate from the root in the chamber and filled with the experimental solution was used as a control blank. Various methods were used to saturate the air within the experimental chamber with water vapor; in some experiments a continuous spray of water was maintained within the chamber without coming into contact with the plant itself; in others vapor-saturated air under a slight positive pressure was slowly passed through, or vessels of distilled water were placed on the chamber floor preceding the experiment and the interior was not disturbed during the experiment. Similar results were obtained by all three methods. Between readings, the fog on the thin glass window was removed by a special wiper manipulated from the outside. A relatively constant room temperature ($\pm$ 0.5° C.) was maintained throughout an experimental period. Excised roots were maintained in position by attaching them to a fine glass rod with a minute quantity of vaseline; this rod was attached to the potometer support. Experiments were run in quadruplicate. Volume error in rates due to readings was less than 0.002 mm.^{3} per mm.^{2}

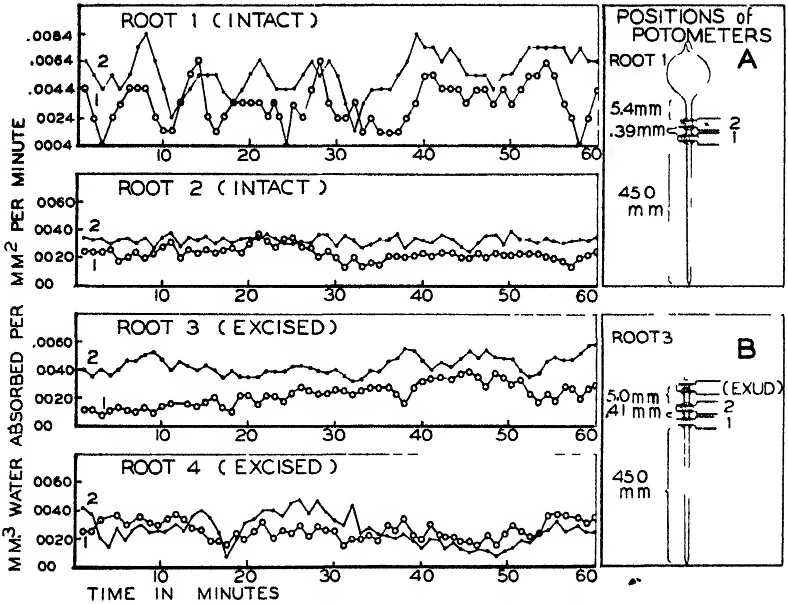


FIG. 1. Volume of distilled water absorbed per unit surface per unit time by two contiguous local zones of tissue in the basal regions of two intact and two excised roots in a vertical position. Diagrams A and B show positions of potometers 1 and 2 on roots 1 and 3. The potometers on roots 2 and 4 were in the same relative positions. Curves 1 and 2 for each root show the rates of intake at the corresponding potometer contacts. Roots were placed in the chambers 3 hours before observations were made. The roots were 4 days old. Room temperature, 26° C.

WATER INTAKE BY CONTIGUOUS REGIONS DURING SHORT INTERVALS OF TIME

Comparisons of the velocities of water absorption in two adjacent regions with relatively similar structural differentiation were made by placing two adjoining potometers at basal levels in both intact and excised roots of the same age and length. Diagrams A and B, figure 1, show the respective positions of adjoining potometers on two roots; curves of the rates of intake of distilled water at each of the potometer contacts in two intact and two excised roots are presented in figure 1. The curves are typical. Most roots, intact and excised, including many which absorbed nutrient solutions from adjoining potometers, manifested rhythmic variations in the rates of intake per minute; the magnitude of fluctuations varied from root to root, and sometimes a general drift in the velocity of absorption at each contact was observed. In most roots (less than 50 mm. in length and 3 or 4 days old), including those represented in figure 1, the rate at the proximal potometer contact was higher and the variations at the two contiguous contacts in each root appeared to occur quite independently of one another.

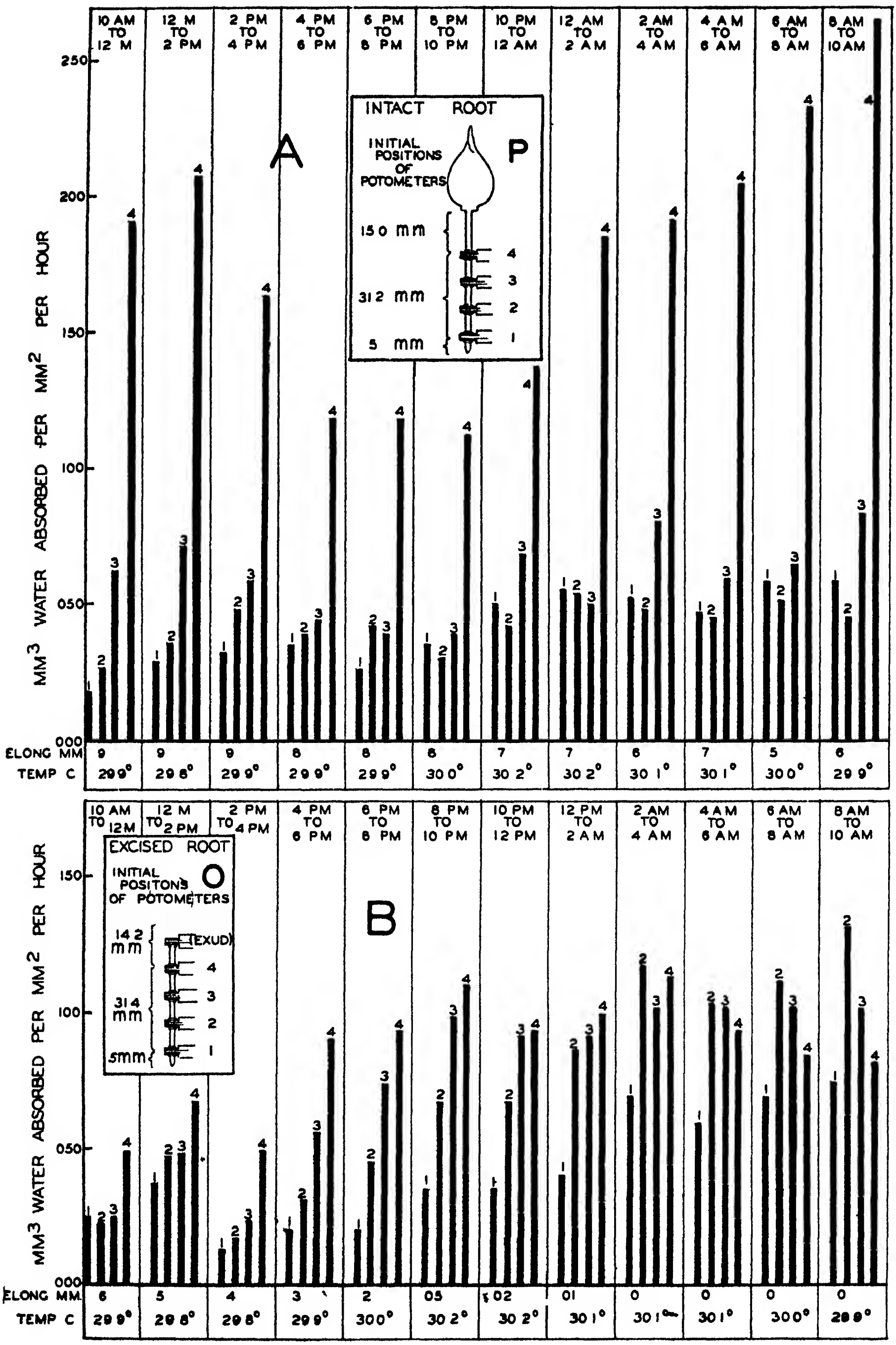
Comparison of rates of water intake in different intact and excised roots during 24-hour periods

In order to compare variations in the axial distribution of the velocity of absorption of intact and excised roots, readings were made at two-hour intervals throughout a 24-hour period. A two-hour time interval was selected in part because apical regions manifest comparatively slow rates; volume intake per unit surface area per hour was calculated for each interval.

The onion bulbs with attached roots were placed in the experimental chambers the evening preceding each experiment. They were supplied with abundant water and aeration. At 8 A.M. the following morning, without removing the glass chamber covers, the potometers were refilled and their positions adjusted and the roots in two of the chambers were excised. Each root was maintained in a vertical position and threaded through potometers 10 m. apart. (See diagrams P and O, figs. 2 and 3). Exudate was collected in the top potometer in two chambers which contained excised roots. No readings were made until 10 A.M.

Sixteen experiments were run. Since uniform results were obtained throughout, data from only four roots present the facts adequately; data showing the axial distribution of rates in two roots (one intact, the other excised) from an experiment run at a temperature of 30 ± 0.2° C. are presented in graphic form in figure 2, A and B, and from another experiment at 23.5 ± 0.2° C. in figure 3.

A comparison of the heights of the vertical bars during the first interval (10 A.M. to 12 M.) show that all four roots exhibited the highest rate of intake at a relatively basal level when the experiment began. With the exception of the excised root shown in figure 2, B, this region continued to maintain the highest velocity of intake throughout the 24-hour period; the maximum in root B, figure 2, appeared at a lower level after 2 A.M. The maintenance of a strict unidirectional gradient of distribution of velocities from interval to interval was not exhibited by any of the four roots considered above; this was also true in the other roots observed but not included in the figures. In both intact and excised roots the gradient varied from interval to interval, sometimes showing one or more minor peaks with a maximum at 30 mm. (from the apex) or above; the pattern of axial distribution thus exhibited a state of flux which was determined by the fluctuations that appeared in each region. These fluctuations in a given root are evident when the heights of the bars with a given number are compared. Comparison of the rates at neighboring regions show that they sometimes exhibited fluctuations opposite in direction, one manifesting an increase and the other a decrease during the same interval of time. The magnitude of


A
INTACT ROOT
INITIAL POSITIONS OF POTOMETERS
P
150 mm
312 mm
5 mm
MM3 WATER ABSORBED PER MM2 PER HOUR
10 AM TO 12 M
12 M TO 2 PM
2 PM TO 4 PM
4 PM TO 6 PM
6 PM TO 8 PM
8 PM TO 10 PM
10 PM TO 12 AM
12 AM TO 2 AM
2 AM TO 4 AM
4 AM TO 6 AM
6 AM TO 8 AM
8 AM TO 10 AM
ELONG MM 9 9 9 8 8 8 7 7 6 7 5 6
TEMP C 29 9° 29 8° 29 9° 29 9° 29 9° 30 0° 30 2° 30 2° 30 1° 30 1° 30 0° 29 9°
B
EXCISED ROOT
INITIAL POSITONS OF POTOMETERS
O
142 mm
(EXUD)
314 mm
5mm
MM3 WATER ABSORBED PER MM2 PER HOUR
10 AM TO 12 M
12 M TO 2 PM
2 PM TO 4 PM
4 PM TO 6 PM
6 PM TO 8 PM
8 PM TO 10 PM
10 PM TO 12 PM
12 PM TO 2 AM
2 AM TO 4 AM
4 AM TO 6 AM
6 AM TO 8 AM
8 AM TO 10 AM
ELONG MM 6 5 4 3 2 05 02 01 0 0 0 0
TEMP C 29 9° 29 8° 29 8° 29 9° 30 0° 30 2° 30 2° 30 1° 30 1° 30 1° 30 0° 29 9°

change during consecutive intervals was not uniform in the different regions of a single root.

The roots absorbed water at higher rates during the latter half of each experiment. Elongation in the excised roots ceased during the latter half of the 24-hour period but continued in the intact roots as indicated by the rates given below the vertical bars in each figure. Since the potometers were not moved throughout the experiments, the position of each relative to the tip "migrated" towards the base. The same tissue remained at each contact all the time, but since elongation occurred at the apex, the regions became more basal relative to the apex. "Migration" of the tip away from the potometers was greater in the intact root and therefore this may account for the steeper gradient sometimes observed in intact roots (*cf.* gradients of roots A and B, fig. 2). Since high rates at basal levels occur in young roots as a rule (**13**), this increase is to be expected in intact roots at this stage of development. The change in rate with time was not synchronous in the different root regions of intact and excised roots; different regions of the same root manifested maxima at different intervals. Although elongation had ceased in excised roots, they continued to absorb water at rates higher than those manifested during the first 10 hours after excision. In one or two cases not represented by figures, maximum rates were observed during the first half of the 24-hour period in both intact and excised roots. Whether or not excised roots exhibit periodicity was not determined.

The range of variation in rates from minima to maxima in intact and excised roots throughout the 24-hour period was greater in excised roots in most cases. Minimal and maximal rates at each region in 12 roots observed during the 24-hour period are shown in figure 4. The ratio $\frac{\text{maximum}}{\text{minimum}}$ given below each set of two vertical bars shows the magnitude of increase at the regions designated by the numbers above the bars. Diagrams of the exact positions of the potometers on each of the 12 roots are not given owing to lack of space.

The greatest magnitude of increase exhibited by an intact root is repre-

FIG. 2. Comparison of the axial distribution of rates of water intake in four different regions of an intact and excised root during two hour intervals throughout a 24-hour period. Roots 5 days old; grown in tap water; same medium in potometers. A. Velocity of absorption in *intact* root; B. Velocity of absorption in *excised* root. Initial positions of potometers in each case are shown by the inset diagrams P and O. Height of each vertical bar gives average rate of absorption in a given region during a two-hour interval; each region is indicated by the number above each bar which corresponds to the number of the potometer in that region in the diagrams P and O. In each case the bar which represents the rate at the potometer in the most distal position (nearest the apex) is at the left, the others follow in order. Elongation in millimeters and the average temperature during each interval are given below the bars.

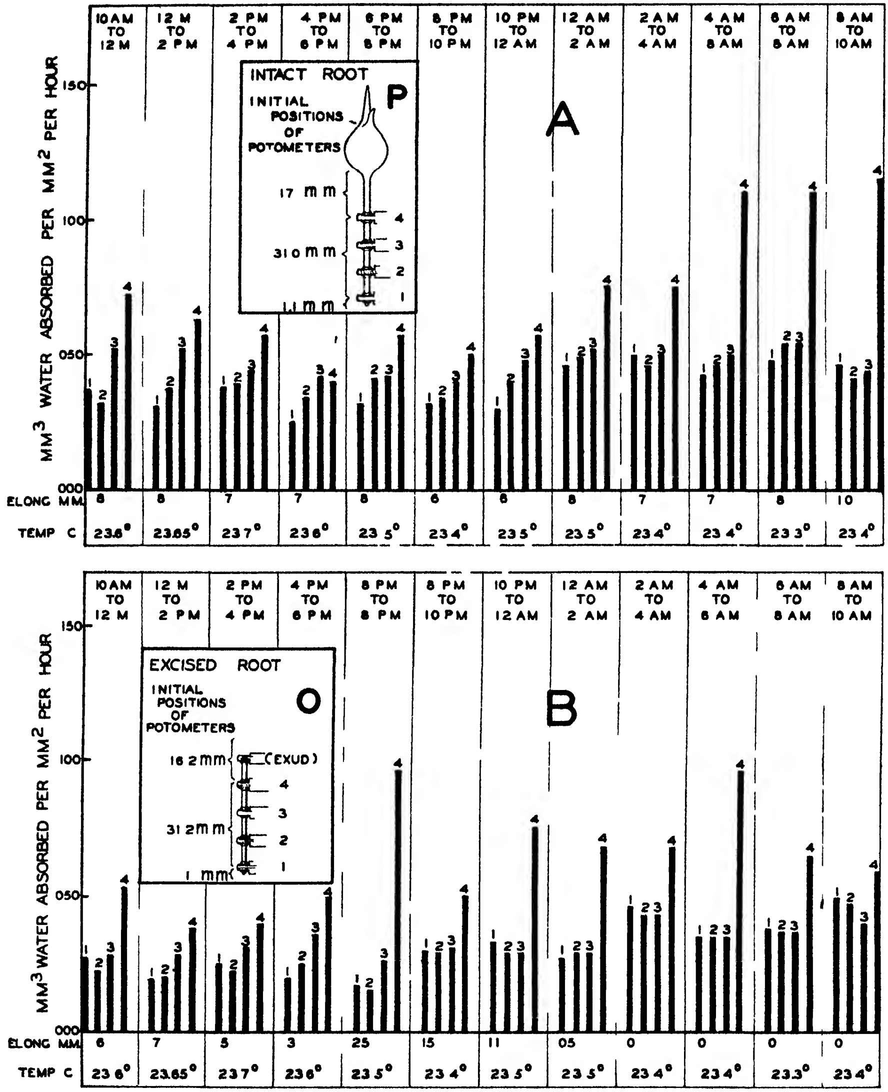


FIG. 3. Legend as for fig. 2.

sented by the ratio of 5 97 (change in rate of 600 per cent. which occurred in root II, figure 4, at potometer 3, at approximately 25 mm. from the apex[2]), a 5-fold increase occurred in two other intact roots (fig. 4, roots I and VI). The minimum increase exhibited by intact roots of this group is

[2] Rate of elongation and the consequent "migration" of each potometer from its initial position was not the same in all roots.

represented by the ratio of 1.35 in the case of root IV, potometer 3 (approximately 25 mm. from the apex).

On the other hand, the excised roots show ratios ranging from maxima of 11.5 and 10.5 (25- and 35-mm. levels of root VIII, fig. 4) to a minimum of 1.65 (at approximately 25-mm. regions of roots IX and X, fig. 4). Although a 3-fold increase at any one level was observed in only one of the 6 intact roots (root III), increases of this magnitude occurred at one or more levels in five of the excised roots.

In general the magnitude of increase in volume flow with time was greater in excised roots; but the absolute velocity of absorption in the different root regions was usually greater in intact roots, especially in the relatively more basal regions. Exceptions occurred in which excised roots manifested higher rates than intact roots of the same experiment.

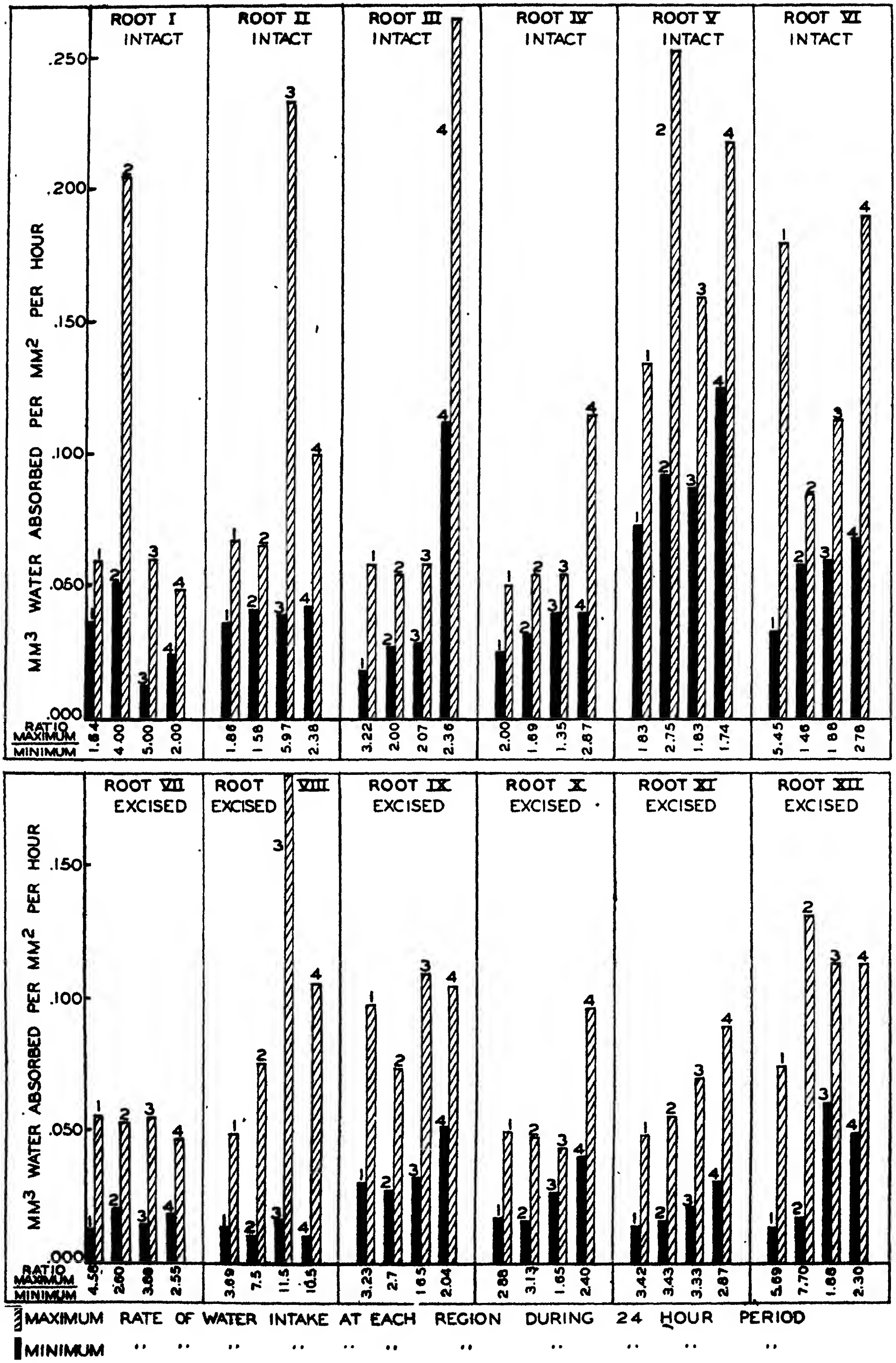
These experiments show that the polar axial gradient of distribution of velocities of intake of water exhibited by intact roots is an inherent characteristic of the isolated root removed from all bulb influence; the experiments also demonstrate the occurrence of oscillating rates in volume flow at different root regions in both intact and isolated roots but a general increase in volume flow in each region with time. With respect to water absorption marked differences in the behavior of different intact and isolated roots appear to be absent.

The data indicate that local regions of any one root exhibit characteristic rates of absorption which depend upon the nature of the local tissue in question. This is true both when attached to the bulb and after isolation from the bulb by cutting as substantiated in the following section.

Effect of presence and absence of bulb on water intake by the same root

The above experiments on different intact and isolated roots did not show whether or not absorption in one or more local regions of the same root is visibly affected when the root is cut from the bulb. Consequently, experiments were run in which measurements of the rates of intake were made at given levels of the same root both preceding and following excision.

Uniform results were obtained in all the experiments; typical data from experiments with potometers in relatively (1) apical and (2) basal positions are shown in figures 5 and 6 respectively. The initial positions of the potometers relative to the apex are shown in the corresponding diagrams A and B. Five or six potometers were used on each root; exudate collected in the top potometer (no. 6, fig. 5, and no. 5, fig. 6) after excision. Clean cut excision of each root without disturbing the menisci of the lower potometers was made by placing an excess of culture solution around the root region at the top potometer and deftly cutting the root with a sharp piece


ROOT I INTACT
ROOT II INTACT
ROOT III INTACT
ROOT IV INTACT
ROOT V INTACT
ROOT VI INTACT
MM3 WATER ABSORBED PER MM2 PER HOUR
.000
.050
.100
.150
.200
.250
RATIO MAXIMUM / MINIMUM
1.64 4.00 5.00 2.00
1.86 1.58 5.97 2.38
3.22 2.00 2.07 2.36
2.00 1.69 1.35 2.87
1.83 2.75 1.83 1.74
5.45 1.46 1.88 2.78
ROOT VII EXCISED
ROOT VIII EXCISED
ROOT IX EXCISED
ROOT X EXCISED
ROOT XI EXCISED
ROOT XII EXCISED
MM3 WATER ABSORBED PER MM2 PER HOUR
.000
.050
.100
.150
RATIO MAXIMUM / MINIMUM
4.58 2.60 3.88 2.55
3.69 7.5 11.5 10.5
3.23 2.7 1.65 2.04
2.88 3.13 1.65 2.40
3.42 3.43 3.33 2.87
5.69 7.70 1.88 2.30
MAXIMUM RATE OF WATER INTAKE AT EACH REGION DURING 24 HOUR PERIOD
MINIMUM " " " " " " " " " " " "

of thin razor blade attached to a long glass rod manipulated from the outside.

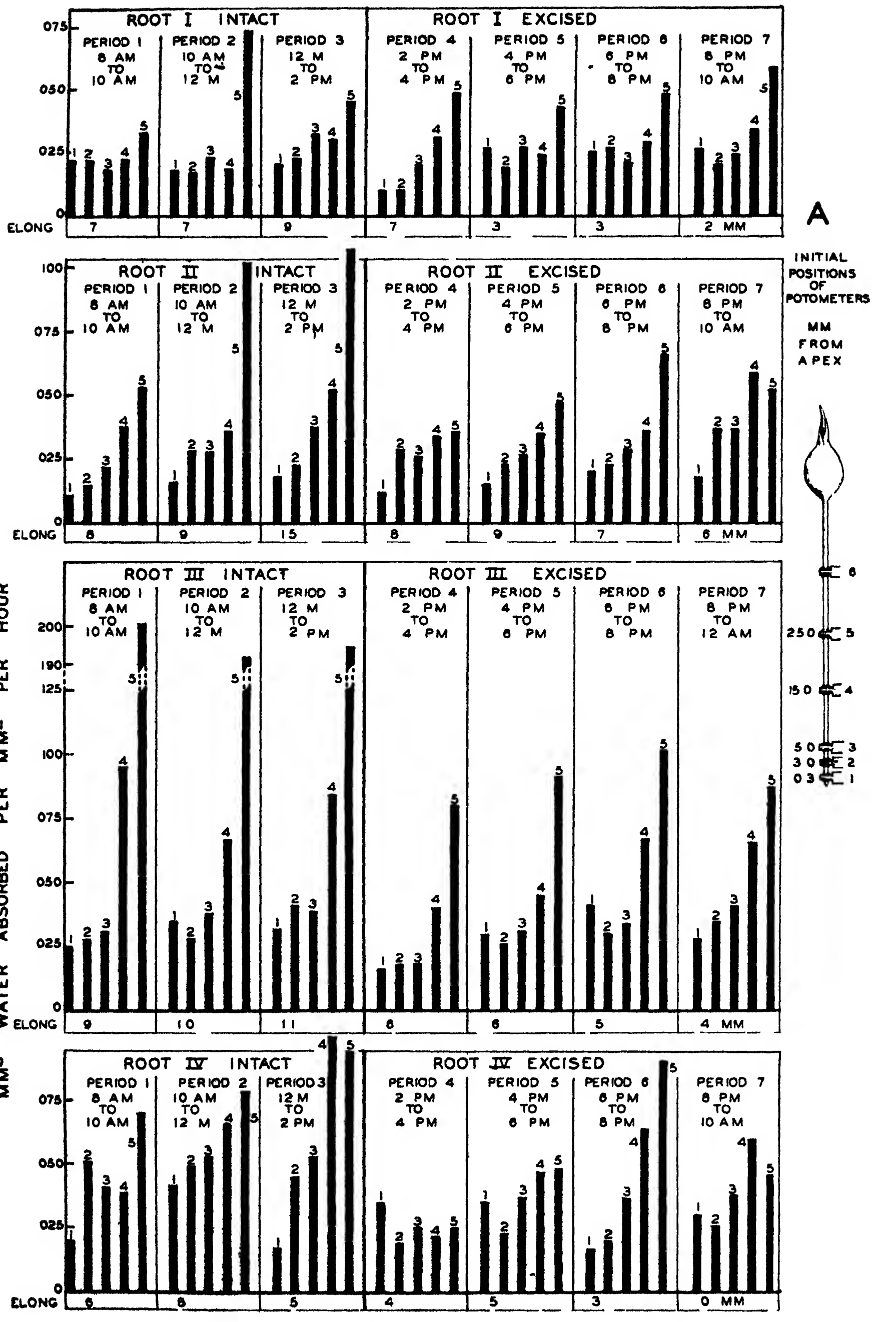
The data represented by figure 5 were obtained from experiments with readings at 2-hour intervals for 6 hours preceding and 6 hours following excision. The average rates during a subsequent overnight period of 14 hours were also determined.

A comparison of the axial gradients of distribution in figure 5 shows that the polar apical-basal differences exhibited when the bulb was present *were maintained in its absence;* the magnitude of the apical-basal difference, however, was less in three of the roots after excision. When the rates manifested in period 3 immediately preceding excision are compared with those of period 4 immediately following, both an increase and decrease in rates at different regions are noted. The decrease in rates was conspicuous at potometers 3, 4, and 5 but root I showed a slight increase in volume flow at potometer 4; an increase in rates was also manifested at potometer 1 in root IV, and at potometer 2 in root II.

It is interesting to note that these changes in rates at local regions between periods 3 and 4 were not necessarily any greater in magnitude nor different in direction from the fluctuations which appeared between two other consecutive intervals, either in the presence or absence of the bulb; compare, for example, the magnitude and direction of change at potometer 5 root 1, and at potometer 1 root IV, during periods 1, 2, and 3 when the bulb was present. Furthermore, increase in volume flow during subsequent periods (periods 5, 6, 7, fig. 5) was exhibited by one or more regions in the absence of the bulb; measurements of rates of intake made immediately following excision are not necessarily representative of the rate of intake in the excised state as shown in the previous section.

At all levels in root V (fig. 6) the average rate for 6 hours preceding was greater than that for 6 hours following excision, and an immediate drop occurred at each potometer contact except one (number 4 at 46.4 mm. from the apex). Decreased volume flow at all potometer contacts was also exhibited by root VI immediately after cutting from the bulb. On the other hand, the total volume intake in root VII (fig. 6) increased after the root had been isolated, although the region at potometer 3 maintained a higher

FIG. 4. Comparison of the minimal and maximal rates of water intake exhibited in different regions of 12 different roots during a 24-hour period. Vertical bars indicated by number 1 represent rates within the first 5 mm. (from the apex); bars designated by the numbers 2, 3, and 4 represent the rates at regions respectively 10, 20, and 30 mm. more proximal than the position of potometer 1. Solid black bars show minimal rates at each region; bars with crossed lines, the maximal rates, in the same regions. The magnitude of increase in each region is given by the ratio $\frac{\text{maximum}}{\text{minimum}}$ directly below the corresponding bars.


ROOT I INTACT
ROOT I EXCISED
ROOT II INTACT
ROOT II EXCISED
ROOT III INTACT
ROOT III EXCISED
ROOT IV INTACT
ROOT IV EXCISED
PERIOD 1 8 AM TO 10 AM
PERIOD 2 10 AM TO 12 M
PERIOD 3 12 M TO 2 PM
PERIOD 4 2 PM TO 4 PM
PERIOD 5 4 PM TO 6 PM
PERIOD 6 6 PM TO 8 PM
PERIOD 7 8 PM TO 10 AM
ELONG
MM
WATER ABSORBED PER MM2 PER HOUR MM3
A
INITIAL POSITIONS OF POTOMETERS
MM FROM APEX

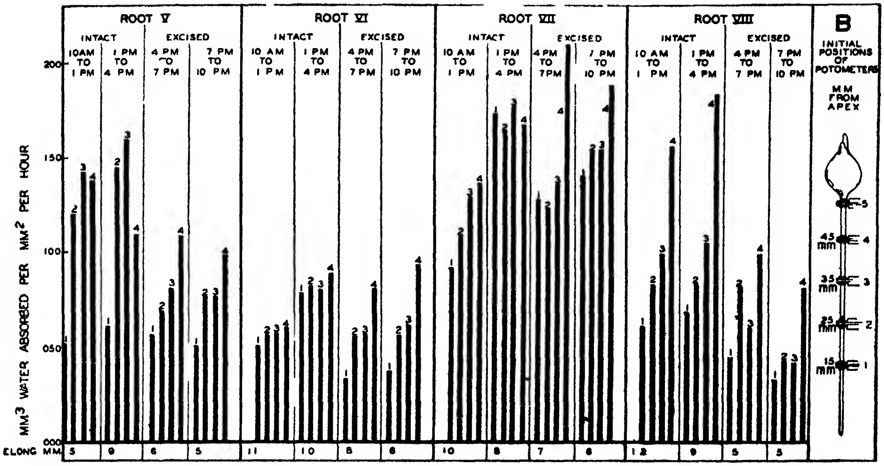


FIG. 6. Comparison of the rates of water absorption at 4 local regions during 6 hours preceding and 6 hours following excision. Initial positions of potometers indicated by diagram B. Initial lengths of roots numbered V, VI, VII, and VIII were 52, 57.6, 59, and 57.5 mm. respectively; age 6 days; grown in TRELEASE culture solution. Placed in chambers night preceding the experiment. Height of each vertical bar represents the rate of water intake at the potometer contact with the corresponding number in diagram B. Room temperature, 25° C. ± 0.5° C.

intake before excision; in this root the axial gradient of distribution, exhibited during a three-hour interval immediately following isolation of the root, was steeper than that exhibited in the 3-hour period immediately preceding, when the bulb was present. There was considerable slowing down of the velocity of absorption in root VIII (fig. 6) with a consequent flattening of the longitudinal gradient of distribution of rates.

The experiments discussed above represent typical results obtained from measurements on 32 roots. When comparisons were obtained between the average rate of water intake during the 6-hour period preceding, and the 6-hour period following excision, seven of the roots displayed an increase in volume flow during the second 6-hour period when the bulb was absent. The experiments show that the longitudinal gradient of velocity of absorption in a saturated atmosphere is usually flattened by excision owing to greater decrease in rates at relatively more basal levels after isolation; but

FIG. 5. Comparison of the rates of water absorption at 5 local regions during 3 intervals preceding and four following excision. Initial positions of potometers relative to the apex indicated in diagram A. Initial lengths of roots numbered I, II, III, and IV were 51, 48.3, 49, and 47 millimeters respectively; age 4 days; grown in TRELEASE solution; same medium in potometers. Placed in chambers night preceding the experiment. Height of each vertical bar represents the rate of intake at the potometer contact with the corresponding number in diagram A. Room temperature, 25° C.

they also demonstrate that volume flow at a single local region and even at all regions may increase immediately after the root has been isolated.

Absorption of water by roots in an inverted position with respect to gravity

In order to determine whether or not higher rates were observed at higher levels because of vertical orientation of the root, experiments were run on both intact and excised roots when inverted.

The bulbs, including short leaves if present, were fitted in an inverted position into perforated corks placed on the floor of each chamber and the inverted roots carefully threaded through the potometers without injury. The length of the root, the positions of the potometers, and duration of the experiments were varied. Consistent results were obtained in all the experiments; typical data are given in figure 7. Rates were first determined in the intact roots for a short period of 3.5 hours and a subsequent period of 10 hours; each root was then cut from the bulb without removing the chamber covers and rates again determined during two periods of different duration.

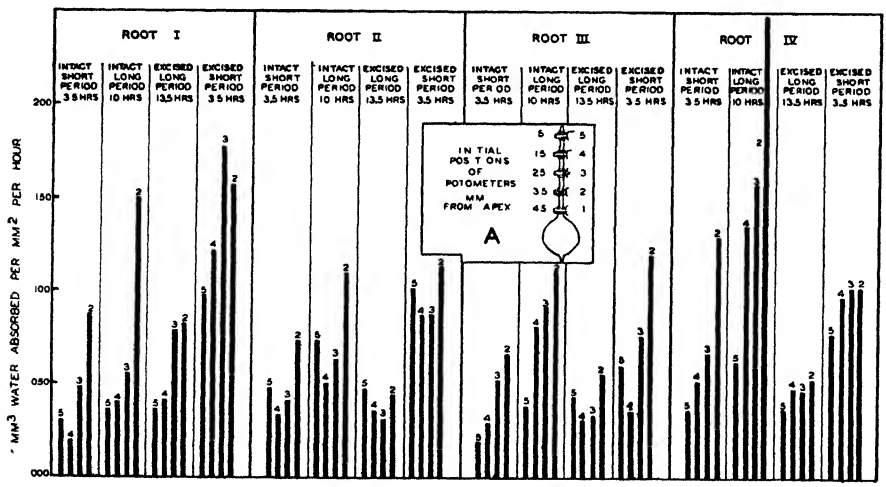


FIG. 7. Comparison of distribution of rates of intake before and after excision in 4 roots, oriented in an inverted position with respect to gravity. Initial positions of potometers shown by inset diagram A. Vertical bars represent rates; numbers above correspond to potometers in diagram A. Rates during four consecutive periods are shown; first reading 8 A.M. Age of roots, 5 days; initial lengths 51 to 54 mm.; grown in tap water; same medium in potometers. Room temperature, 24.5° C.

Comparisons of the data show that in both the intact and excised state, lowest velocities of intake appeared at the apical regions which in this case withdrew water from the highest potometers. A strict unidirectional gradi-

ent of distribution of rates was maintained by roots III and IV during the two periods preceding excision; following excision the steepness of the gradient was less and a minor peak appeared at potometer 5 (near the apex) in root III. Minor peaks in the gradient also appeared in roots I and II both before and after excision (at potometer 5 during the first 3.5-hour period in the intact root and at potometer 3 in the excised root). An increase in rates with time was manifested by the different levels both in the presence and absence of the bulb; in roots I and II the average rates observed during the second period (short period of 3.5 hours) in the absence of the bulb were greater than that observed at corresponding levels during the two periods in the presence of the bulb.

These experiments furnish further evidence that local regions along the root axis manifest independent characteristics whether or not the root is attached to the shoot in a saturated atmosphere.

Water intake at contiguous regions in dead roots

Intact and excised roots which had been killed showed both liquid loss from, and liquid intake by, a local region at a single potometer contact but during different intervals. Table I shows typical results obtained from experiments during which the roots were quickly killed by dipping them into boiling water or applying a jet of steam. The roots were sectioned and examined after the experiments.

Although liquid loss appeared at different levels in the dead intact root A and the dead excised root B, both roots showed greater water uptake than liquid loss during the 10-hour experimental period; the uptake by the excised root was much less than that of the intact root. Comparisons of rates at the same levels in the same root in the presence and absence of the bulb show that root C manifested a marked increase in water uptake at 3 levels and liquid loss at one level after it had been killed, root D exhibited a decrease in water uptake at basal levels, but no liquid loss; after excision water uptake practically ceased.

No water uptake occurred in dead intact roots when the period of exposure to heat was longer than two seconds; in such cases pronounced liquid loss from the root occurred at each level and the root flattened into a thin ribbon.

The experiments showed that dead root tissues in either the intact or excised root are leaky systems with respect to water uptake; in both cases a given level may manifest water influx simultaneous with liquid loss in an adjacent region.

Discussion

In onion roots (*Allium cepa*) water intake is a continuous flux process but with changing velocity. The different rates of intake are sharply local-

TABLE I

EFFECT OF KILLING ROOTS ON WATER INTAKE BY DIFFERENT LOCAL REGIONS
RATES ($mm.^3/mm.^2/hr.$) OF WATER INFLUX DESIGNATED BY "+" AND OF LIQUID LOSS DESIGNATED BY "−" SIGNS

Root A	Data from *intact* root killed with hot water					
		Positions of potometers; mm. from apex				
	Interval	5	15	25	35	
	8–10 A.M.	−0.201	−0.086	+0.030	−0.014	
	10 A.M.–12 M.	+0.073	+0.034	0.000	+0.187	
	12–2 P.M.	−0.091	−0.033	+0.046	+0.045	
	2–4 P.M.	+0.146	+0.069	+0.029	+0.072	
	4–6 P.M.	−0.009	+0.051	+0.006	+0.004	
Root B	Data from *excised* root; killed with hot water before excision					
		Positions of potometers; mm. from apex				
	Interval	3	10	20	30	
	8–10 A.M.	0.000	+0.025	−0.114	+0.026	
	10 A.M.–12 M.	0.000	+0.003	+0.204	−0.019	
	12–2 P.M.	0.000	−0.011	+0.110	0.000	
	2–4 P.M.	−0.008	0.000	−0.022	+0.049	
	4–6 P.M.	−0.010	+0.020	0.000	+0.005	
Root C	Data from single root *before* and *after* killing; steam applied to root only					
		Positions of potometers; mm. from apex				
	Interval	4	14	24	34	44
Intact living	8–9:30 A.M.	+0.025	+0.021	+0.023	+0.036	+0.040
	9:30–11 A.M.	+0.029	+0.036	+0.027	+0.049	+0.067
Intact dead	12–1:30 P.M.	+0.083	+0.120	+0.149	+0.148	−0.162
	1:30–3:00 P.M.	+0.082	+0.089	+0.094	−0.398	−0.410
Excised dead	3:00–4:30 P.M.	+0.022	+0.002	+0.013	−0.049	no exudation
	4:30–6:30 P.M.	−0.012	0.000	−0.027	+0.018	no exudation
Root D	Data from single root *before* and *after* killing; steam applied to root only					
		Positions of potometers; mm. from apex				
	Interval	6.5	16.5	26.5	36.5	46.5
Intact living	9–11 A.M.	+0.033	+0.047	+0.116	+0.119	+0.110
	11 A.M.–1 P.M.	+0.030	+0.037	+0.102	+0.125	+0.106
Intact dead	2–4 P.M.	+0.053	+0.040	+0.55	+0.075	+0.72
	4–6 P.M.	+0.050	+0.066	+0.79	+0.075	+0.70
Excised	6–8 P.M.	+0.025	+0.032	−0.270	+0.015	no exudation
	8–10 P.M.	+0.021	+0.001	0.000	+0.021	no exudation

ized at different levels at any one instant. With respect to time and space, water absorption involves a shifting pattern of rates along the longitudinal axis. More knowledge of cell dynamics is necessary to explain what factors

alter the flux equilibria of contiguous cells similar in structure, and further experimentation is necessary to reveal how adjacent regions in the root often manifest simultaneous positive and negative accelerations in water influx under constant external conditions in both intact and excised states. Are such oscillations characteristic of adjacent "spots" on the surface of a single cell—does the spacial pattern of dynamic forces in the cell vary from instant to instant causing simultaneous restriction of intake at one "spot" and acceleration at another (*cf.* LUND, **9**)? Studies in the electrophysiology of onion roots show that spontaneous variations of electric potentials occur in adjacent regions under constant external conditions (**1, 9, 10, 12, 14**). From his experiments on Douglas fir (**8**) LUND concluded that the inherent electric energy output might function in electroendosmotic flow. Whether or not there is any interrelation between spontaneous variations of bioelectric potentials of the root and water intake or transport is not known at present. Fluctuations of ion uptake by single plant and animal cells have been reported by BROOKS (**2**).

The fact that the basal regions of roots less than 65 mm. in length and less than a week old continue to absorb greater quantities of water per unit surface, even after excision in a saturated atmosphere, indicates that the same inherent difference in machinery operating to maintain this apical-basal difference before excision continues to exist after excision, and in this respect is fundamentally independent of the shoot-root relationship. This does not imply that the shoot-root relationship does not also enter in as a condition because the absolute rates of intake in the basal regions are in *most* cases immediately lowered by excision. It may be that the difference (before and after excision) in basal regions represents a "pull" by the shoot which in this instance acts as a "suction pump." *In some roots, however, the basal regions may absorb even greater quantities of water after* excision —in that case the difference may represent a "push" or the action of a "force pump" in the root tissue; but we have no critical evidence which isolates the "push" and "pull" forces that might be operating in the root tissue *per se*. It may be that the demands of the bulb and growing leaves are greater at some times than at others, and differ in different roots, so that a greater water deficit actually exists in some roots before excision, and, furthermore, it may be that some roots contain greater amounts of nutritional reserve. Water deficits in excised roots would hardly be expected to exist after the roots manifested vigorous exudation; yet the rates of intake were greater when possible deficits were supposedly satisfied. The quantity of water absorbed immediately after excision does not represent the maximum absorbing capacity of the root tissue because the rates increase with time after excision under the given conditions. Removal of the bulb and growing leaves in a saturated atmosphere may remove an accessory suction

force but the data show that the unit *volume of transport through a given level in an excised root may be just as large or larger than when it had the added force of the bulb to supplant its own in the transport of water.*

PREVOT and STEWARD (11) demonstrated that the entire surface between the apex and emergence of secondary roots of 12-day-old barley plants represented a potential absorbing surface for salts. They also showed a pronounced longitudinal gradation in the capacity of salt accumulation, and that segments near the apex attain higher concentrations than those more remote. Since the roots were not only a different type, but were also slightly older than the roots discussed in the present paper, strict comparison of results cannot be made; but it is interesting to note that opposite gradients occur; in the excised barley root the peak of salt intake for a given interval is within the first 15 mm.; in the excised onion root the peak of water intake is beyond this region and near the base. If the gradient of ion intake in the onion root is similar to that of barley this would mean greater dilution of surrounding solution at the apex during a given interval since ion intake would be relatively greater and water intake relatively less than in basal regions. Experiments are under way to determine whether or not this occurs.

Death of the tissues destroys the mechanism *regulating* water intake in both intact and excised roots; water uptake in a dead root occurs by an entirely different mechanism. The dead excised root which no longer manifests exudation at the basal end exhibits siphon action and leaks at one or more levels; the dead intact root which may serve as a wick to a greater or less extent also leaks at one or more levels; the wick action immediately after death may involve greater or less uptake than in the living state; some dead roots in both the intact and excised state manifest no water uptake whatsoever. It is not known whether or not liquid loss occurred in the dead roots of KRAMER's experiments (7) because he was interested in transpiration rates and did not measure water intake. KRAMER maintains (7, p. 484) that "in experiments with sunflower, tomato, and tomato plants it was found that in most cases during the first twenty-four hours after the roots were killed the plants with dead roots transpired only about one-half as much water per unit of leaf area as the plants with living roots."

The living root is not merely a piece of machinery which interposes resistance to water, neither is it merely a sieve or a wick with regard to transpiration and the transport of water. The living root in position can deliver more water than that same root when dead. In a saturated atmosphere it manifests a characteristic flux gradient of water intake which is maintained by that root when it is upright or inverted, intact or excised, but which disappears when it is killed, liquid loss appearing at one or more levels. There is no cessation of water influx in the healthy living intact and

excised root; although changes in flux rates occur in contiguous regions, there appears to be a continuous overall drive of water through the root tissues. Before operative details of the working machinery of water absorption can be forthcoming we need further accurate accounts of the rates of volume transport in different types of roots, old and young, under carefully controlled conditions.

Attention should be called to the point that the objective has been to isolate the mechanism of transport of water into the root and reduce the problem to characteristics of absorption at a small element of root surface. This cellular mechanism has a large but variable volume capacity for transport of water across it and in this respect the problem is fundamentally the same as that of the uriniferous tubule in the animal.

Summary

1. By means of a technique previously described (**13**) simultaneous measurements of the rates of water absorption by contiguous regions were made in a saturated atmosphere on single intact and excised onion roots (*Allium cepa*) 65 mm. or less in length and less than a week old.

2. The experiments on intact and excised roots of corresponding age, length, and history carried out under comparable conditions of humidity, temperature, and light showed similar characteristics of behavior in water absorption with respect to (a) localization of the highest rates in relatively more basal regions; (b) an over-all increase of rates at each level with time throughout 12 two-hour intervals (24-hour period); and (c) fluctuations of rates in contiguous regions and in the axial gradient of distribution under constant external conditions.

3. The rates of water influx at contiguous levels of the same root after excision were equal to, greater, or less than before excision.

4. Both intact and excised roots which had been killed showed pronounced irregularities of water intake in contiguous regions; both liquid loss and uptake occurred at different levels during the same interval. The living root in position can deliver more water than that same root when dead.

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THE DETERMINATION OF SELECTED CHEMICAL CHARACTERISTICS OF SOIL WHICH AFFECT THE GROWTH AND COMPOSITION OF PLANTS

G. R. Noggle and F. Lyle Wynd

Introduction

It is obvious that very great specialization of modern botanical science has led to many important developments in this field of knowledge which otherwise would not have been possible. But these very advances which specialization makes possible are often obtained at the price of a narrowing of the field of intellectual outlook by the workers who are most concerned. Ultimately, the workers themselves become hampered in their studies by the intensity of their specialization, and the discoveries and techniques of adjacent sciences which they could profitably use are too frequently ignored.

At no place in botanical science has this intensity of specialization produced greater harm along with its gains than in the study of plant physiology and agronomy. Unfortunately, in many American educational institutions the main distinction between these subjects seems to be based on the idea that plant physiology deals with unimportant aspects of unimportant plants and that significant plants lie in the province of agronomy. There can be a justifiable distinction between these subjects only in the matter of emphasis rather than in the actual subject matter, for certainly the plant physiologist is less concerned with the ultimate economic significance of his work than is the agronomist. But in so far as the plant physiologist and the agronomist are dealing with the actual growth and behavior of plants it is difficult indeed to avoid the conclusion that if there is a distinction at all between their work it is without a difference.

A specific example of the results of an artificial and pedantic distinction between these two sciences, is their different approach to the problem of the mineral nutrition of plants. The plant physiologist too frequently visualizes the problem as involving primarily a mixture of ions dispersed in a liquid. His approach, therefore, centers on artificially controlled liquid nutrient media. The agronomist frequently visualizes the problem as one depending almost exclusively on colloidal phenomena in naturally occurring soils. Each of these viewpoints has led to valuable results, and no serious fault can be attributed to the different attitudes themselves. The fault lies only in the fact that these specialized viewpoints, each based on vast fields of literature, aggregations of specific data, and highly specialized techniques, tend to lead research workers to a biased interpretation of the major problem and many significant aspects are either confused or ignored.

The plant physiologist is particularly at fault, since he has permitted

his attack on the problem of the mineral nutrition of plants by the artificial solution procedure, which was pressed so vigorously a few years ago, to come to a rather ignominious end in so far as fundamental knowledge of the growth of plants in soil is concerned. The really significant aspects of the problem have now been taken over by the agronomist, who is centering his attention on the colloidal phenomena occurring in soils. This approach is leading to rapid progress. It is the belief of the writers, however, that even further progress would be made if the strict plant physiologist would revive his interest in the mineral nutrition of plants modified by the recent development in agronomy and especially by applying to his own theoretical problems many of the techniques worked out by the agronomist.

Like all fields of study, the literature of agronomy presents a vast and confusing aspect; it presents a serious problem to even the most ambitious plant physiologist. The purpose of the present paper is to describe in considerable detail a selected group of techniques that are available for the study of a few of the characteristics of soil which are of special significance in the growth and composition of plants. Only chemical and colloidal characteristics are included which might reasonably be expected to vary throughout the growing season, in different seasons, and with different fertilizer treatments.

The analytical procedures are based on several types of soil extracts. How to obtain a soil fraction, or extract, that is truly related to plant nutrition is one of the most perplexing problems in the study of soil fertility. The present uncertainty as to the proper procedure necessitates the several types of extractions described. In most instances, air dry samples of soil are used.

The laboratory should be equipped with an electric drying oven, steam bath, chemical balances, glass electrode, mechanical shaker, sieves, centrifuge, distillation equipment, Kjeldahl digestion equipment, bank of suction filters, filter rack, digestion hood, hot plate, colorimetric equipment, and a carbon combustion train.

The usual details of chemical technique must be observed at all times. The capital letters enclosed in parentheses in the diagrams of the schematic separations refer to the procedures in the later portion of this paper which describe the determination of the specific ions indicated.

Collection and preparation of field samples

The variability of soils makes it almost impossible to devise an entirely satisfactory method of collecting truly representative field samples. The details of any particular procedure must be modified to suit the particular purpose for which the sample is desired. Remove from the surface all vegetable materials not intimately incorporated into the soil. For the

examinations described in the present paper, the sample should be representative of the soil layer to the average depth of the most recent plowing. Ordinarily this will be about seven inches. Sometimes it may be desirable to collect samples from various strata to a depth of 40 inches or more by means of a soil auger. When using a soil auger, enlarge the diameter of the first boring before penetrating below the plowed depth in order to prevent the contamination of the lower samples by falling fragments from the upper layers.

A sufficient number of samples should be collected so that a satisfactory composite sample may be prepared. After the material has been air dried, reduce the lumps by rubbing in a porcelain mortar, or by any method that will not reduce the rock fragments themselves. Sift through a sieve having circular openings 1 mm. in diameter, thoroughly mix, and store in a stoppered container. If the total quantity of any component is to be determined, finely pulverize a part of the sample prepared as above (**1**).

Calculation of results

The results of chemical determination on soil may be expressed in a variety of ways. When expressed as pounds per acre, it is first necessary to determine the weight under field conditions of a given volume of soil from a small area to a depth of 7 inches or to the depth of the most recent plowing. This method is especially valuable in the making of comparisons between soil conditions and crop production per acre. Results also may be expressed in terms of percentages of the air-dry or oven-dry sample. This latter procedure may be used more appropriately when soils are being studied comparatively or when changes in the soil are being followed.

The Roman numerals and headings in subsequent paragraphs indicate separate sub-samples of the composite field sample. The procedures for the examination of each of these sub-samples is described under such headings.

Water relationships

I. Moisture

Weighing bottles and their stoppers are heated 6 to 12 hours in an electric oven at 105° to 110° C. Cool in a desiccator over calcium chloride and weigh to $\pm$ 0.001 gm. The weighing bottles should be handled with a small piece of cheese cloth or with crucible tongs. Transfer 2 to 5 gm. of the fresh, undried field sample of soil to each weighing bottle by means of a porcelain spatula, weigh, and dry in the electric oven from 8 to 12 hours. Cool in a desiccator and reweigh. Clay soils and those containing an appreciable amount of organic matter should be dried for an additional 8 to 12 hours and reweighed.

Moisture determinations should be made in triplicate and the results expressed as percentages of the oven-dry sample.

II (A). WATER HOLDING CAPACITY

Dry the weighing bottles and their stoppers in the electric oven from 6 to 12 hours at 105° to 110° C., cool in a desiccator over calcium chloride, weigh to ± 0.001 gram, and store in the desiccator until needed. Carefully fit a no. 1 filter paper into the bottom of a Hilgard soil cup and fill level to the edges with soil. Care should be taken to prevent pressing or compacting of the sample. Place the filled cup in a shallow pan and add water until the cup is nearly submerged. As soon as saturation is complete, remove the cup, cover with a watch glass and allow to drain at room temperature over night. Transfer the sample to a weighing bottle and weigh. Then dry in the electric oven from 8 to 12 hours. Clay soils and those rich in organic matter should be dried for an additional 8 to 12 hours. Cool in the desiccator and reweigh.

The determinations should be made in triplicate and the results expressed as the percentage of water on the basis of the oven-dry sample which the soil can hold against the force of gravity.

II (B). PERCENTAGE OF THE WATER HOLDING CAPACITY UNDER FIELD CONDITIONS

The percentage of the water holding capacity under field conditions is calculated from the data obtained in I (A) and II (A).

$$\frac{\text{Moisture in fresh sample}}{\text{Water holding capacity}} \times 100 = \text{percentage of the water holding capacity}$$

Acidity relationships

III. pH

Shake 100 gm. of air-dry soil for 1 min. with 250 ml. of carbon-dioxide-free water in a 1-liter Erlenmeyer flask. Decant a portion of the supernatant liquid into a 150-ml. beaker and determine the pH by the glass electrode (**11**). If the glass electrode is a sufficiently rugged type, it may be immersed directly into the soil suspension. If the soil suspension reaches its pH equilibrium slowly, it may be advisable to allow the suspension to stand for 1 hour before the determination is made.

IV. LIME REQUIREMENT

Add 5 gm. of air-dry soil which has been sifted through a 10-mesh sieve to each of 4 test tubes. Add 1 gm. of potassium chloride and 25 ml. of distilled water to each tube. To one tube, add 5 ml. of 0.00124 per cent. $Na_2CO_3 \cdot H_2O$; it corresponds to 1 ton of calcium carbonate per acre of 2,000,000 pounds of soil. Add to successive tubes 2, 3, and 4 times this amount. If desired, the steps may be made 2.5 ml. per tube which corresponds to one-half ton applications of lime per acre. Shake vigorously

about 25 times and let settle. Add 3 drops of brom-thymol blue indicator and agitate just enough to mix the indicator with the upper half of the solution. The first tube in the ascending order of carbonate additions which shows a green to greenish-blue color is considered to have received adequate calcium. The calculated amount of calcium carbonate required per acre must be corrected for impurities in the commercially obtained product (**4**).

If the samples exhibit wide variation in the lime requirement, the preliminary test with potassium thiocyanate solution (4 per cent. in 95 per cent. alcohol) will permit the lime requirement to be determined with a minimum number of sodium carbonate additions. Mix a small amount of the air-dry soil with an equal volume of the potassium thiocyanate solution. Shake well and allow to stand for 10 min. If no color develops, the soil is not acid; increasing degrees of color indicate progressively a higher acidity. The addition of sodium carbonate may be made corresponding to 1, 2, and 3 tons of lime per acre for slightly acid soils and 3, 4, and 5 tons per acre for more acid soils.

Colloidal relationships

V (A). Total base exchange capacity

Weigh out 10 gm. of the air-dry sample and treat with 50 ml. of neutral 1.0 N ammonium acetate in a 250-ml. beaker, and allow to stand for 1 hour with occasional stirring (5). Transfer to a 9-cm. Buchner filter funnel and wash the soil with successive small additions of ammonium acetate until 450 to 500 ml. of leachate have been obtained. Before starting the leaching, be certain that the filter paper is wet and that the suction has been turned on before the mixture of soil and ammonium acetate solution is transferred to the filter. If the suction is not being applied at all times, the filter paper will curl and permit some of the soil to go into the leachate. Transfer the leachate to a 1-liter beaker and preserve for the determination of the total replaceable bases as described in section V (B), where it is referred to as "leachate A."

Wash the soil free from excess ammonium acetate by washing the sample on the filter with successive additions of methyl alcohol, which has been made neutral to brom-thymol blue by a drop by drop addition of concentrated ammonia, until 450 ml. of alcohol have been used. Care should be taken to wash down the sides of the filter funnel. Remove the filter flask and replace with a clean flask. Then leach the soil with 400 ml. of 0.1 N hydrochloric acid. The procedure must not be interrupted until this process of releasing the replaceable ammonium ions with hydrochloric acid has been completed. Transfer the leacheate, which now contains the replaceable ammonia, to an 800-ml. Kjeldahl flask, add 5 gm. of sodium hydroxide pellets and distill into a 500-ml. Erlenmeyer flask containing 50 ml. of standard 0.1

N hydrochloric acid until the volume of liquid remaining in the distillation flask has been reduced to 60 to 80 ml. Titrate the remaining hydrochloric acid with standard 0.1 N sodium hydroxide, using brom-thymol blue as an indicator.

The milliliters of standard 0.1 N hydrochloric acid used is equal to the base exchange capacity in terms of milliequivalents per 100 gm. of dry soil. The determinations should be made in triplicate.

V (B). Total replaceable bases

Evaporate to dryness on the steam bath, the ammonium acetate leaching, now designated as "leachate A," which was obtained in the procedure V (A). Transform the acetate salts of the basic ions present to carbonates by igniting the dry residue to whiteness on a silica plate. Cool, add 50 ml. of standard 0.1 N hydrochloric acid and 25 ml. of water. Heat to boiling and allow to boil for 15 seconds. Cool, and titrate the remaining hydrochloric acid with standard 0.1 N sodium hydroxide, using brom-thymol blue as the indicator (5).

The milliliters of standard 0.1 N hydrochloric acid used to react with the carbonates is equal to the total replaceable bases in the soil in terms of milliequivalents per 100 gm. of dry soil. The determinations should be made in triplicate. The titrated solution should be designated as "solution B" and preserved and used for determination of individual replaceable bases as described in section V (E).

V (C). Percentage base saturation

The percentage of base saturation of the field samples may be calculated from the results obtained by the procedures of sections V (A) and V (B).

$$\frac{\text{Total replaceable bases}}{\text{Base exchange capacity}} \times 100 = \text{Percentage of base saturation}$$

V (D). Replaceable hydrogen

The replaceable hydrogen may be calculated at this point by substracting the percentage of base saturation from 100. This method is correct in principle but is subject to many errors. See section VI for a direct method of determining replaceable hydrogen.

V (E). Individual replaceable bases

The individual basic ions may be determined on suitably prepared aliquots of the neutralized solutions obtained by the procedure described in section V (B) and designated there as "solution B." Acidify the "solution B" with 5 ml. 0.1 N hydrochloric acid and evaporate to dryness on the steam bath. Dissolve the residue with water and make up to volume in a 100-ml. volumetric flask.

An aliquot of 50 ml. is used for the determination of potassium. The remaining aliquot of 50 ml. is used for the determination of calcium, magnesium, and also potassium if desired. Ordinarily, however, more accurate determinations of potassium may be made on a separate aliquot.

Aliquot 1

$Co(NO_2)_3 \cdot 3NaNO_2$

precipitate K (D)[1]

filtrate

Aliquot 2

Remove Fe group (A)

NH_4OH

precipitate Fe, Ti, Al, P

filtrate $(NH_4)_2C_2O_4$

precipitate Ca (B)

filtrate $(NH_4)_2HPO_4$

precipitate Mg (C)

filtrate K if desired (D)

[1] The letters enclosed in parentheses refer to the procedures described under the same letters in the text.

VI. Exchangeable hydrogen by titration

The titration procedure of determining exchangeable hydrogen is to be preferred over the difference method described in section V (D). Place 5 or 10 gm. of air-dry soil in a Gooch crucible and leach with 250 ml. of neutral, 1 N barium acetate adjusted to pH 7. The leachings are then titrated with standard 0.1 N barium hydroxide to pH = 7.00. The final end point should be determined by the glass electrode. For an original sample of 10 gm., the milliliters of barium hydroxide used equals the milliequivalents of replaceable hydrogen per 100 gm. of air-dry soil (**14**).

VII. Organic colloid base exchange capacity

Weigh out 10 gm. of the air dry sample and treat with 80 ml. of 15 per cent. hydrogen peroxide in a 400-ml. beaker. Cover with a watch glass and digest for 1 hour on the steam bath. Remove the watch glass and evaporate to dryness. In some instances a second digestion with hydrogen peroxide may be necessary to destroy the organic matter. When the sample is free from organic matter, determine the base exchange capacity as de-

scribed in section V (A). The value obtained will be the base exchange capacity of the inorganic colloids, and the difference between the total base exchange capacity determined by the procedure described in section V (A) and the inorganic colloid base exchange capacity is equal to the organic base exchange capacity (**13**).

Nitrogen

VIII. Total nitrogen

Place 10 gm. of the air-dry field sample in an 800-ml. Kjeldahl flask with 50 ml. of concentrated sulphuric acid and 2 gm. of commercial salicylic acid and allow to stand for 30 min. with frequent shaking. Add 10 gm. of sodium thiosulphate and heat gently for 5 min., cool, and add 10 gm. of salt mixture. This salt mixture consists of 20 parts potassium sulphate or anhydrous sodium sulphate, 2 parts ferrous sulphate, and 1 part copper sulphate. Digest until clear or very nearly so, cool, dilute with 150 ml. of water; cool, and add 75 ml. of 45 per cent. sodium hydroxide by carefully pouring down the neck of the flask so that the alkali forms a layer in the bottom of the flask. Connect to the still, shake, and distill about 150 ml. into 50 ml. of standard 0.1 N hydrochloric acid contained in a 300-ml. Erlenmeyer flask. Titrate the remaining acid with standard 0.1 N sodium hydroxide, using brom-thymol blue as an indicator. Each ml. of 0.1 N acid neutralized by the ammonia in the distillate corresponds to 1.401 mg. of nitrogen. Express the result as a percentage of the air-dry sample (**1**).

IX (A). Ammonia

Ammonia usually is determined on the fresh field sample before it has been dried (**18**).

Weigh 100 gm. of the sample into a 500-ml. Erlenmeyer flask, add 100 ml. of 2 N potassium chloride solution, and sufficient 2 N hydrochloric acid to bring the mixture to the proper acidity; add sufficient water to bring the total volume of liquid added up to 200 ml. In order to determine if the mixture has been adjusted to the proper acidity, a separate 10-gm. sample of soil is shaken with 10 ml. of 2 N potassium chloride, 1 ml. of 2 N hydrochloric acid, and 9 ml. of water in a 125-ml. Erlenmeyer flask and filtered. If the filtrate is bright red with the thymol blue indicator (pH about 1.5), sufficient acid has been added. If the indicator color is orange or yellow (pH about 2 or more) additional 2 N acid must be added until the proper color is obtained. Then add 10 times this amount to the sample which is to be analyzed for ammonia. Most soils, provided that they are free from calcium carbonate and not too rich in humus, will assume the proper acidity with 100 ml. of 2 N potassium chloride, 10 ml. of 2 N hydrochloric acid and 90 ml. of water. Add about 7 drops of toluene. If a considerable amount

of carbonate is present, shake the flask by hand until most of the carbon dioxide has escaped. Stopper tightly and shake for 1 hour on the mechanical shaker. Transfer the entire contents of the flask to a 16-cm. fluted filter funnel with a single pouring. Discard the first 20 to 30 ml. of the filtrate, collect the remainder in a 300-ml. Erlenmeyer flask, and stopper tightly until used for the determination of ammonia.

A 100-ml. aliquot is used for the ammonia distillation. This represents one-half of the original sample if 200 ml. of liquid have been added to it. A strictly accurate dilution factor would necessitate a correction for the variable volume of acid added and also for the amount of water originally in the sample. Since a single 100-ml. aliquot of the filtrate is used, it is not necessary to consider the volume of liquid remaining with the soil residue or on the filter paper. The aliquot to be used for distillation is transferred to an 800-ml. Kjeldahl flask and 200 ml. of water and 5 gm. of magnesium oxide are added. Distill about 150 ml. into a 300-ml. Erlenmeyer flask containing 50 ml. of standard 0.02 N hydrochloric acid. The residue in the distillation flask is preserved for the determination of nitrate as described in section IX (B). The unneutralized acid is titrated with 0.02 N sodium hydroxide, with brom-cresol green as the indicator until the color matches that of an acetate buffer at pH 4.8 to which the indicator also has been added. This buffer solution is prepared by adding 6 ml. of 0.2 N sodium acetate solution and 4 ml. of 0.2 N acetic acid to a 300-ml. Erlenmeyer flask and diluting to the approximate volume of the distillate; then add 1 ml. of 0.04 per cent. brom-cresol green indicator. A few drops of saturated mercuric chloride will preserve the buffer from contamination by microorganisms for at least a month.

The weight of the original fresh field sample is corrected for the water content and the results expressed as the percentage of ammonia, and of nitrogen in the air-dry soil. Blank determinations should be run and the proper correction applied. Sometimes sufficient carbon dioxide develops from the magnesium oxide that it is advisable to boil the filtrate before titration.

IX (B). NITRATE

The residue remaining in the Kjeldahl flask after the distillation of ammonia as described in section IX (A) is made up to 300 ml. with water and about 2.5 gm of powdered Devarda's alloy added. Because of the excessive frothing that may occur, it is advisable to connect the flask to the distillation equipment and allow to stand over night before distilling and even then the distillation should proceed slowly so that 200 ml. of distillate will collect in about 1.5 hours. The distillate is titrated as described in section IX (A) and the results expressed as the percentage of nitrate, and of nitrogen, in the air-dry material (**18**).

IX (c). Organic nitrogen

The organic nitrogen is calculated by subtracting the sum of ammonia and nitrate nitrogen from the total.

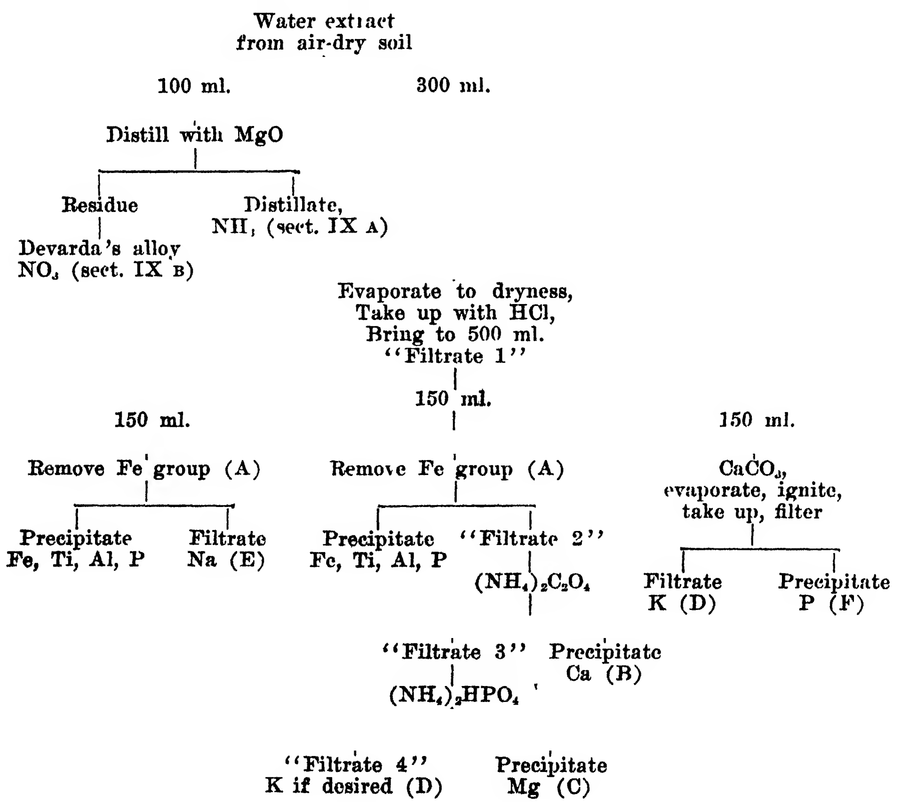
Extracts

X. Water extract

Shake 100 gm. of the air-dry soil with 500 ml. of water in a 1-liter Erlenmeyer flask for 4 hours on the mechanical shaker. Filter on a 9-cm. Buchner filter funnel using a fine filter paper.

An aliquot of 100 ml. of the filtrate which represents 0.2 of the original sample is treated with magnesium oxide for the determination of ammonia as described in section IX (A) and for nitrate as described in section IX (B).

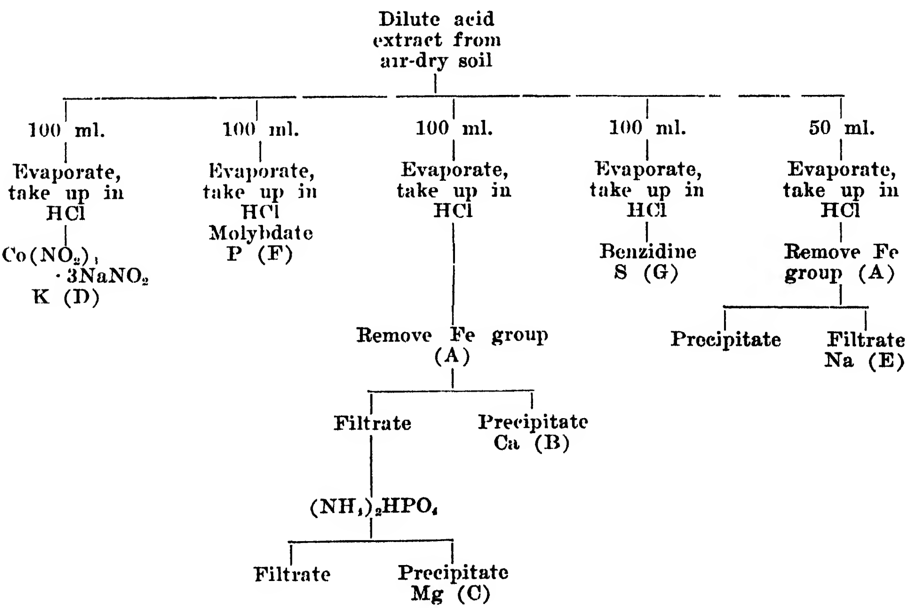
An aliquot of 300 ml. is placed in a 1-liter beaker, 10 ml. of concentrated hydrochloric acid are added, and then evaporated to dryness on the steam bath. Take up the residue with dilute hydrochloric acid (1:3) and filter through no. 42 filter paper. Collect the filtrate in a 500-ml. volumetric flask and wash the residue with hot water until 500 ml. of filtrate have been



obtained. After cooling, the filtrate is brought back to volume with cold water. This solution is designated as "filtrate 1." One aliquot of 150 ml. is freed from the iron group (A) and used for the determination of sodium (E). Another aliquot of 150 ml. is freed from the iron group (A) and used for the determination of calcium (B), and magnesium (C). A third aliquot of 150 ml. is used for the determination of potassium (D) and phosphorus (F). This later aliquot is prepared as follows (**12**): Add 0.5 gm. of calcium carbonate to the aliquot and evaporate to dryness on the steam bath and ignite until the chlorides of iron, aluminum, and magnesium have been converted into oxides. During the ignition lumps are broken up with a glass rod. The ignition is complete when a clear solution is obtained on the addition of water. Cool the ignited residue, take up with hot water, and filter. The chloride salts of the alkali metals are obtained in the filtrate in which the potassium can be determined by the cobaltinitrite method (D). The residue is digested with dilute sulphuric acid and filtered. The residue is washed with hot water and the phosphorus determined in the combined filtrate and washings by the phosphomolybdate method (F). Each of the three aliquots contain 0.18 of the original sample.

XI. Dilute acid extract

A sample of 50 gm. of air-dry soil is mixed with 500 ml. of water and 5 gm. of citric acid in a 1-liter Erlenmeyer flask and shaken 24 hours on a



mechanical shaker (7). If carbonates are present in a significant quantity, an additional equivalent amount of citric acid is used. Filter on a Buchner filter funnel. Pipette 100-ml. aliquots into each of 5 beakers. Each aliquot contain 0.2 of the original sample. Acidify each aliquot with 5 ml. of concentrated hydrochloric acid, evaporate to dryness on the steam bath, and gently incinerate to insure the dehydration of silica. The residue is dissolved in dilute hydrochloric acid (1:3), and evaporated to dryness on the steam bath. The residue is again taken up with dilute hydrochloric acid and filtered. The various ions are then determined as indicated above.

Carbon

XII. Carbonate

Pulverize the air-dry sample until it passes a 60-mesh sieve so that the acid may react with crystals of calcite that may be included among quartz crystals. For soils low in carbonate use samples of 10, 25, or 50 gm.

Arrange an absorption train so that the current of air passes through 2 scrubbing bottles containing 10 per cent. sodium hydroxide before it enters the flask which contains the sample. The air enters the evolution flask through a small, stoppered separation funnel. After passing through the sample flask, purify the air by first passing through a tower containing a suspension of silver sulphate in a 5 per cent. sulphuric acid solution, then through concentrated sulphuric acid, and finally through a tube of Dessichlora or Anhydrone. The carbon dioxide is absorbed by Ascarite in a Fleming absorption bulb. The drying chamber of the absorption bulb should contain the same drying agent that was used in the tower preceding the Fleming bulb. Finally, the air is passed through a sulphuric acid tower to protect the Fleming bulb from moisture and back pressure.

Add the sample to the Erlenmeyer evolution chamber and draw air through the system for 5 minutes. Weigh the Fleming bulb and replace it in the train. Close the stopcock of the separation funnel, disconnect its stopper from the train and place 60 ml. of 10 per cent. hydrochloric acid containing 5 per cent. of stannous chloride. Connect the stopper of the separation funnel with the absorption train and apply suction to the system. Slowly open the stopcock and allow the acid mixture to run into the evolution flask. Aerate and agitate for 1 hour with an air current of 3 to 4 bubbles per second.

When soils have been treated with magnesite or dolomite, when they have been derived from a magnesite parent rock, or when it is from glaciated areas which contain considerable quantities of transported dolomite, it may be necessary to apply heat after the initial evolution of carbon dioxide has subsided. When the heat-evoked evolution has subsided, remove the heat and continue the agitation and aeration for 20 min.

Reweigh the absorption bulb and report the carbonate carbon as carbon dioxide or carbon (**1**).

XIII (A). Organic carbon combustion procedure

Arrange a combustion train so that the stream of oxygen passes through two towers containing 10 per cent. potassium hydroxide, through a mercury safety valve, and then into the combustion tube over the boat containing the sample (**1**). There follows, within the furnace, an 8-inch core of loosely packed platinized asbestos. On leaving the combustion tube, the gas passes through a tower of concentrated sulphuric acid, a tower of 40-mesh granulated zinc, a tube of Dessichlora or Anhydrone, a Fleming absorption bulb containing Ascarite, and finally a Fisher bubble counter containing sulphuric acid as a protection against back pressure and water vapor. The drying compartment of the Fleming bulb should contain the same dehydrating agent that preceded the Ascarite in the train. An asbestos-filled copper coil with a wire handle is placed in each end of the combustion tube to protect the rubber stoppers from the heat of the furnace.

Bring the furnace to a temperature of 900° to 950° C. Adjust the flow of oxygen from the cylinder by a needle valve and after 10 min. weigh the Fleming bulb. Weigh 2 gm. of air-dry soil, mix with 2 gm. of finely divided potassium dichromate, place in an alundum boat, and place within the heated zone of the combustion tube. Aerate with an oxygen stream for about 4 hours. Reweigh the Fleming bulb and calculate the increase in weight due to carbon dioxide in terms of total carbon or as organic carbon by subtracting the carbonate carbon determined by the procedure described in section XII.

XIII (B). Titration procedure

The air-dry sample is ground in a mortar until it passes a 100-mesh sieve. Iron or steel mortars must not be used. Place a sufficient amount of the sample to contain 10 to 25 mg. of carbon in a 300-ml. Erlenmeyer flask. Add 10 ml. of standard N potassium dichromate from a burette, and follow with 20 ml. of concentrated sulphuric acid. Shake for 1 minute, cool, and dilute to about 150 ml. Add 5 gm. of sodium fluoride. Titrate the remaining chromic acid with standard 0.4 N ferrous ammonium sulphate. One ml. of ½ per cent. diphenylamine is used as an indicator. If the end point is passed, the solution may be back titrated with the standard potassium dichromate.

Since only about 76 per cent. of the carbon is accounted for by the observed reduction of the chromic acid, a correction factor of 1.32 must be applied. One ml. of normal potassium dichromate equals $1.32 \times 3.0 = 4.0$ mg. of carbon. Report as total carbon or as organic carbon by subtracting the carbonate carbon determined as described in section XII. This pro-

cedure is of limited accuracy and it should not be used except for rough comparisons of carbon content (**16**).

XIV. Loss on ignition

Weigh about 2 gm. of the air-dry soil into a porcelain or platinum crucible and dry at 100° C. for 24 hours; weigh and ignite to redness, with occasional stirring, for 4 hours. When considerable amounts of carbonate are present, cool, moisten with a saturated solution of ammonium carbonate; dry and heat to 200° C. for 30 min. Cool in a desiccator and report the loss in weight as organic matter in the oven dry soil (**1**).

Analytical procedures

A. Removal of iron group

Add ammonium hydroxide, drop by drop, to the acidified solution until the precipitate which forms requires several seconds to dissolve. The ammonium hydroxide must be free from carbonates. The solution should remain faintly acid. Heat nearly to the boiling point and add ammonium hydroxide to a slight excess. The precipitate, which consists of iron, aluminum, titanium, phosphorus, etc., is boiled for a few minutes in a covered beaker to expel excess ammonia and to coagulate the gelatinous precipitate. A slight amount of ammonia must be present, and if no odor of ammonia can be detected, add a small amount drop by drop. Do not allow the precipitate to settle, but stir and pour on a filter; wash with hot water. Return the precipitate and the filter paper to the original beaker; macerate the filter paper with a stirring rod and make the volume up to approximately 150 ml. Dissolve the precipitate with a few drops of dilute hydrochloric acid and reprecipitate with ammonium hydroxide as described above. The combined filtrates and washings from the 2 precipitations may be used for the determination of calcium, magnesium, sodium, and potassium.

B. Determination of calcium

The filtrate and washings obtained after the removal of the iron group is made slightly ammoniacal if not already so (**9**). Heat to boiling, and add hot 4 per cent. ammonium oxalate with constant stirring until no more precipitate forms. There should be an excess of ammonium oxalate present. Boil for 2 min., cover with a watch glass, and digest on the steam bath for ½ hour; cool for 2 hours. Filter and wash with 5 successive portions of 10 ml. of cold, neutral, saturated calcium oxalate (**3**). The filtrate and washing from the oxalate precipitation are preserved for the determination of magnesium as described in section C. Wash the oxalate precipitate through the filter paper with hot 2 N sulphuric acid and the oxalic acid is titrated with

standard 0.1 N potassium permanganate. Each ml. of 0.1 N potassium permanganate is equal to 0.002 gm. of calcium.

C. Determination of magnesium

Volumetric procedure.—The determination of magnesium may be carried out on an aliquot of 100 ml. of the original acidified stock solution of the unknown prepared for analysis from the soil extract and freed from the iron group as described in section A or on the combined filtrate and washings obtained from the calcium precipitation described in section B.

Add 5 ml. of concentrated hydrochloric acid and methyl red indicator. Dilute to 150 ml. and add 10 ml. of a saturated solution of diammonium hydrogen phosphate. Add ammonia of specific gravity 0.88 slowly with constant stirring until the solution is neutral. Continue stirring for about 5 minutes, and then add an additional 5 ml. of ammonia and continue stirring for about 10 min. Allow to stand for at least 4 hours or preferably over night. Filter, and wash with ammonia solution which contains 3 to 5 per cent. by volume of ammonia. In some instances it may be desirable to determine potassium at this point in which case the filtrate and washings from the ammonia precipitation should be preserved. Transfer the precipitate to a dry 12.5-cm., no. 42 filter paper and allow the bulk of the moisture to be absorbed. After 3 to 5 min. transfer the precipitate to a second filter paper for additional drying. In some instances, a third drying will be necessary. Preserve the filter papers that have been used to dry the precipitate and allow to dry at room temperature or place them on the grating of a drying oven at a temperature of 50° to 60° C. After 15 to 20 min. in the oven or 45 min. in the air, transfer the filter papers and the precipitate to a 400-ml. beaker. Add standard 0.1 N sulphuric acid in a known amount until the papers are disintegrated and the precipitate is dissolved. Add 2 drops of a 0.1 per cent. alcoholic solution of methyl orange. If the solution is only faintly pink, add 5 ml. of standard 0.1 N sulphuric acid. Dilute to about 100 ml. and titrate with standard 0.1 N sodium hydroxide until the appearance of a clear yellow color (8).

One ml. of N/10 sulphuric acid = 0.0024 gm. of Mg.

Gravimetric procedure.—Concentrate the combined filtrate and washings obtained from the calcium precipitation described in section B on the steam bath to about 100 ml. and add cautiously 20 to 30 ml. of nitric acid. Evaporate to dryness, cover the beaker with a watch glass and gently ignite on a hot plate to remove the ammonium salts. Add 5 ml. of concentrated hydrochloric acid and evaporate nearly to dryness. Dissolve the residue with hot water and a small quantity of hydrochloric acid. If necessary, filter and wash the filter paper with about 100 ml. of hot water. Precipitate the magnesium as magnesium ammonium phosphate by the addition of 3

ml. of a 10 per cent. solution of ammonium phosphate. Add ammonia until the solution is slightly alkaline. Stir vigorously; allow to stand 15 min. Add 15 ml. of ammonia and let stand over night. Filter and wash the precipitate with dilute ammonia (1:9). Transfer the filter paper and the precipitate to a weighed crucible, moisten with ammonium nitrate, dry, and ignite. Cool in a desiccator and weigh as $Mg_2P_2O_7$.

Weight of $Mg_2P_2O_7 \times 0.21843 =$ gm. of Mg.

D. Determination of potassium

Volumetric procedure.—The aliquot for analysis should contain between 2 and 15 mg. of potassium in a neutral aqueous solution of 10-ml. volume. Add 1 ml. of 1 N nitric acid and 5 ml. of trisodium cobaltinitrite solution (1 gm. of trisodium cobaltic nitrite in 5 ml. of water), mix, and allow to stand for 2 hours at a temperature of about 20° C. Filter in a porous-bottomed porcelain crucible, using 0.01 N nitric acid in a wash bottle to make the transfer. Wash 10 times with 2 ml. portions of the 0.01 N nitric acid. Wash the precipitate into a 250-ml. beaker, place the crucible in the beaker, and make to about 100 ml. with water. Add 20 ml. of 0.5 N sodium hydroxide and boil for 3 min. Withdraw into another beaker a slight excess of 0.05 N standard potassium permanganate, make to 50 ml. with water, and add 5 ml. of concentrated sulphuric acid. Pour the hot potassium cobaltinitrite solution into the cold potassium permanganate solution, transfer the crucible and wash the beaker with a small amount of water. Add an excess of 0.05 N standard sodium oxalate solution, heat to boiling, and complete the titration with potassium permanganate. Ml. of $KMnO_4 \times$ normality of $KMnO_4 \times 7.1084 =$ mg. of K in sample (**17**).

Gravimetric procedure.—This procedure requires the potassium salt to be present in a concentration of about 1.5 per cent. in respect to potassium (**6**). A hot solution of the potassium salt in 15 to 25 ml. of water is treated with 25 to 40 ml. of saturated sodium 6-chloro-5-nitro-m-toluenesulphonate. Boil gently for at least 5 min. or until the precipitate dissolves. Add more water if necessary. Let stand for 24 hours at room temperature and filter on a weighed Gooch filter or a sintered glass filter G4. Wash the precipitate with 2 to 3 times its weight of water. Dry to constant weight at 110° C.

If the original sample contains much sodium or other metallic ions, it is advisable to wash the precipitate with a few ml. of a solution of the saturated sulphonate solution rather than with water.

The factor for potassium is 0.1347 times the sulphonate precipitate.

Gravimetric procedure as potassium chloroplatinate.—An ammonium chloride-potassium chloroplatinate solution is prepared by dissolving 100 gm. of ammonium chloride in 500 ml. of water, and adding 5 to 10 gm. of pulverized potassium chloroplatinate and shaking at intervals for 6 to 8

hours. Allow the mixture to settle over night and filter. The residue is preserved and used for the preparation of a fresh supply.

One hundred ml. of the solution from either a dilute acid extract or a total replaceable base determination must be made free from calcium (**1**). The solution is heated in a 250-ml. volumetric flask and a slight excess of ammonium hydroxide is added; add saturated ammonium oxalate until the precipitation is complete. Cool, dilute to volume, and filter through a dry filter paper. Place a 50-ml. aliquot of the filtrate in a Sillimanite evaporating dish and bring nearly to dryness. Add 1 ml. of 50 per cent. sulphuric acid, evaporate to dryness, and ignite to whiteness at a dull red heat. Cool, dissolve in hot water, add 0.5 ml. of 50 per cent. hydrochloric acid and then an excess of chloroplatinic acid.[2] Evaporate on the steam bath to a thick paste. Avoid exposure to ammonia fumes. Treat with successive 10-ml. portions of 80 per cent. ethyl alcohol, and decant on a weighed Gooch crucible. Continue the washing until the alcoholic filtrate is colorless. Then wash the precipitate successively 5 or 6 times with 10-ml. portions of the ammonium chloride-potassium chloroplatinate solution. Wash finally with several portions of 80 per cent. alcohol. Dry the precipitate for 30 minutes at 100° C. and report as K or as K_2O. The factor for K is 0.16085, that for K_2O is 0.19376.

E. Determination of sodium

The uranyl zinc acetate reagent is prepared in 2 parts. Part A is prepared by adding 10 gm. of uranyl acetate, and 6 gm. of 30 per cent. acetic acid to 49 gm. of water. Part B is made by adding 30 gm. of zinc acetate and 3 gm. of 30 per cent. acetic acid to 32 gm. of water. After solutions have been attained by warming, mix them together and allow the solutions to stand 24 hours. Filter to remove the precipitate of uranyl zinc sodium acetate. The filtrate is saturated with the triple salt due to the sodium unavoidably present in the reagents. If the reagent is stored in Pyrex, it will remain clear for long periods (**2**).

An aliquot of 1 ml. of the original stock solution of the unknown is freed from the iron group by the procedure described in section A. The aliquot should not contain more than 8 mg. of sodium. Add 10 ml. of the uranyl zinc sodium acetate reagent, stir and let stand for at least 30 min. Filter by suction through a sintered glass crucible weighed without being heated, and wash 5 to 10 times with 2-ml. portions of the uranyl zinc acetate reagent. After each washing, remove the reagent as completely as possible by washing with 2-ml. portions of alcohol which has been saturated with uranyl zinc sodium acetate. Finally, wash well with ether, and free the precipitate from ether by suction. Allow the crucible to stand at room temperature for 10 min. or longer. The precipitate has the composition

[2] Solution containing 0.2 gm. metallic platinum or 0.42 gm. H_2PtCl_6 per 10 ml.

$(UO_2)_3ZnNa(CH_3COO)_9 \cdot 6H_2O$. The chemical factor for sodium is 0.01495.

If the unknown contains more than 50 mg. of potassium per ml., potassium uranyl zinc acetate will be precipitated. This difficulty may be overcome by dissolving 1 gm. of the sample in 5 ml. of water and then adding a hot solution consisting of 2 gm. of ammonium perchlorate and 3 ml. of water. Then add 25 ml. of 95 per cent. ethyl alcohol; cool and filter. Wash 5 times with 2-ml. portions of 95 per cent. alcohol. Evaporate the filtrate to dryness on the steam bath. Dissolve the residue in 1 ml. of water and proceed with the determination of sodium as described above.

F. Determination of phosphorus

Volumetric procedure.—The ammonium molybdate reagent is prepared by dissolving 100 gm. of molybdic acid in a mixture of 144 ml. of concentrated ammonia and 271 ml. of water. Pour this molybdate mixture slowly with constant stirring into a mixture of 489 ml. of concentrated nitric acid and 1148 ml. of water. Store in a warm place for several days. This reagent improves with age and it is advisable to prepare at one time a sufficient quantity to last for several months. The solution should not be used until it has stood long enough that a portion heated to 40° C. deposits no yellow precipitate. After long standing, decant the supernatant liquid and store in a glass-stopped bottle. Just before using, add 5 ml. of concentrated nitric acid to each 100 ml. of reagent and filter.

The standard sodium hydroxide may be prepared by diluting 323.81 ml. of standard 1 N alkali, free from carbonate, to 1 liter. An aliquot of 100 ml. should neutralize 32.38 ml. of standard 1 N acid. The standard acid is prepared by titrating with the standard alkali.

The solution to be analyzed should contain about 0.02 gm. of P_2O_5 (**1**). Transfer the unknown solution to a 300-ml. Erlenmeyer flask, add 5 to 10 ml. of concentrated nitric acid, and then add concentrated ammonia until the precipitate which forms dissolves slowly with vigorous stirring. Dilute if necessary to 75 to 100 ml. and bring to a temperature of 25 to 30° C. Add sufficient ammonium molybdate reagent to insure complete precipitation of the phosphorus. Shake on the mechanical shaker for 30 min. Decant through a sintered glass filter. Wash the precipitate twice with 25- to 30-ml. portions of water by agitating thoroughly and allowing the precipitate to settle. Finally transfer the precipitate to the filter and wash with cold water until the filtrate from 2 fillings of the filter yields a pink color with the phenolphthalein indicator when 1 drop of the standard alkali is added. Transfer the precipitate and filter into a 400-ml. beaker. Dissolve the precipitate with a measured volume of 0.3238 N standard alkali and titrate the

excess alkali with standard 0.3238 N acid using phenolphthalein as the indicator.

Each ml. of 0.3238 N alkali used in dissolving the precipitate equals 1 mg. of P_2O_5.

COLORIMETRIC PROCEDURE.—The ammonium molybdate-sulphuric acid reagent is prepared by dissolving 25 gm. of ammonium molybdate in 200 ml. of water. Heat to 60° C. and filter. Dilute 280 ml. of concentrated sulphuric acid to 800 ml. When both solutions are cool, add the ammonium molybdate to the sulphuric acid slowly with constant shaking. Cool and dilute to 1000 ml. This final solution is 10 N in respect to sulphuric acid and contains 2.5 gm. of ammonium molybdate per 100 ml.

The stannous chloride solution is prepared by dissolving 25 gm. of stannous chloride ($SnCl_2 \cdot 2H_2O$) in 1000 ml. of 10 per cent. solution by volume of hydrochloric acid. Filter if necessary. Store in a bottle and protect the solution from the air with a layer of white mineral oil 5 mm. thick. The reagent should be removed in drops through a siphon closed with a glass stop-cock.

A standard phosphate solution is prepared by dissolving 0.2195 gm. of potassium dihydrogen phosphate in water and diluting to 1000 ml. This solution contains 50 p.p.m. of phosphorus. Dilute 50 ml. of this solution to 500 ml. to obtain the final stock solution containing 5 p.p.m. The standard colorimetric phosphate solutions are prepared by adding 5 ml. of stock solution to 95 ml. with water in a 150-ml. Erlenmeyer flask, adding 4 ml. of the ammonium molybdate-sulphuric acid solution and 6 drops of the stannous chloride reagent. Shake and dilute to 100 ml. The final solution contains 0.25 p.p.m. of phosphorus. For the determination of very small amounts of phosphorus, use 2 ml. in place of 5 of the stock phosphate solution. The resulting colorimetric standard contains 0.1 part per million of phosphorus. After standing 10 to 12 min., the color of the standard solution begins to fade. Then another drop of stannous chloride reagent will preserve the color for an additional 10 to 12 min.

Aliquots of the water extract of soils may be treated with the reagents directly unless they are colored, turbid, or decidedly acid or alkaline. Color and turbidity should be removed by appropriate means, and the reaction adjusted to neutrality before the reagents are added. In all cases, 4 ml. of the ammonium molybdate-sulphuric acid reagent and 6 drops of the stannous chloride reagent are added to an appropriate aliquot of the unknown and the color compared with the standard within 10 min. A correction is made for the dilution by the reagents (**17**).

G. DETERMINATION OF SULPHUR

VOLUMETRIC PROCEDURE.—An aliquot of the soil extract containing 0.05 to 2.0 mg. of sulphate is pipetted into a 30-ml. Pyrex beaker (**10**). Silica

and porcelain dishes occlude some of the sulphate and are therefore unsuitable. Add 0.5 ml. of concentrated nitric acid and evaporate to dryness on the steam bath. Moisten the residue with a few drops of nitric acid and again evaporate to dryness on the steam bath. Heat the dish and the residue in an electric oven at 400° C. for about 1 hour. The use of gas flames must be avoided since sulphur in the gas introduces positive errors. Cool, moisten with a few drops of nitric acid, and evaporate to dryness on the steam bath. Add 2 ml. of water and 1 drop (0.05 ml.) of 0.1 N hydrochloric acid to the residue. Warm gently on the steam bath and transfer to a tapered, graduated, 10-ml. centrifuge tube. Rinse the beaker 3 times with 0.5 ml. of water and make up the contents of the centrifuge tube to 4 ml. Add 1 ml. of the freshly prepared benzidine hydrochloride solution. The benzidine hydrochloride solution is prepared by dissolving 8 gm. in 1 liter of water and filtering. After 5 min., place the tube in a mixture of crushed ice and water for 10 min. Centrifuge for 5 min. at 3000 r.p.m. Decant the supernatant liquid, and wash the residue with 5 ml. of 80 per cent. alcohol. Again centrifuge for 5 min., decant, wash a second time with 80 per cent. alcohol, and centrifuge. Pour off the supernatant liquid and drive off the remaining alcohol by placing the tube in a beaker of hot water. Add 5 ml. of a 0.5 per cent. solution of potassium hydroxide. After the precipitate has dissolved, transfer the contents of the centrifuge tube to a 125-ml. Erlenmeyer flask. The tube should be rinsed 4 times with 5 ml. of water. Add 1 ml. of concentrated sulphuric acid and heat on the steam bath. When the solution is hot, add standard 0.05 N potassium permanganate from a burette until the apparent end point is reached. Add an excess of 25 per cent. of the volume used, and then an extra 1 ml. Heat the solution on the steam bath for 10 min. Add 2.0 ml. of 0.05 N sodium oxalate. When the precipitated manganese has dissolved, complete the titration with standard permanganate.

The total ml. of 0.05 N potassium permanganate used less 2 and multiplied by 0.118 gives the mg. of sulphate present.

Gravimetric procedure.[3]—Evaporate a 100-ml. aliquot nearly to dryness on the water bath to expel the excess of acid; add 50 ml. of water, heat to boiling, and add drop by drop 5 per cent. $BaCl_2$ solution until no further precipitation occurs. Continue the boiling for about 5 min. and allow to stand in a warm place over night. Decant the liquid on the filter. Wash the precipitate with 15 to 20 ml. of boiling water, transfer to the filter (paper no. 42) and wash free from chlorides with boiling water. Ignite, and weigh as $BaSO_4$.

$BaSO_4 \times 0.13735 = S$.

[3] Association of Official Agricultural Chemists. Methods of Analysis. 1916.

Preparation of indicators for volumetric analysis

Brom-cresol green: Grind 0.1 gm. of the dye in an agate mortar with 1.43 ml. of 0.1 N sodium hydroxide. Dilute to 250 ml. which gives a 0.04 per cent. solution.

Brom-phenol blue: Grind 0.1 gm. of the dye in an agate mortar with 1.49 ml. of 0.1 N sodium hydroxide, and dilute to 250 ml. which gives a 0.04 per cent. solution.

Cochineal: Digest 3 gm. of pulverized cochineal in a mixture of 50 ml. of 95 per cent. alcohol and 200 ml. of water for 2 days at room temperature with frequent shaking, and filter.

Methyl orange: Dissolve 0.1 gm. of methyl orange in a small quantity of alcohol and dilute to 100 ml. with 50 per cent. by volume alcohol.

Methyl red: Grind 0.1 gm. of methyl red in an agate mortar with 3.7 ml. of 0.1 N sodium hydroxide and dilute to 100 ml. with water. The solution may also be prepared by dissolving 0.1 gm. of the dye in 100 ml. of 95 per cent. alcohol.

Phenolphthalein: Dissolve 0.5 gm. of the dye in 50 ml. of 95 per cent. alcohol and dilute with shaking to 100 ml. with water.

Brom-thymol blue: Grind 0.1 gm. of the dye in an agate mortar with 1.60 ml. of 0.1 N sodium hydroxide. Dilute to 250 ml. which gives a 0.04 per cent. solution.

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SIGNIFICANCE OF PHLOEM EXUDATE OF *CUCURBITA PEPO* WITH REFERENCE TO TRANSLOCATION OF ORGANIC MATERIALS[1]

BRUCE J. COOIL

(WITH TWO FIGURES)

Introduction

The green leaves of plants are the major loci of carbohydrate synthesis. Materials necessary for vegetative growth, fruit development, and storage of reserves must be moved from the leaves. That this movement occurs in the phloem has become a matter of general acceptance (**9, 22**). Considerations of the forms of carbohydrate which may account for movement have been restricted largely to the hexoses, glucose and fructose, and the disaccharide sucrose. MASON and MASKELL (**19**) found sucrose to be higher in concentration in the inner bark of the cotton plant than in the outer bark. Sucrose also showed a greater response to ringing than did reducing sugars. From such considerations as well as concentration gradients, they concluded that sucrose was the principal mobile form in cotton. More recently ENGARD (**12**) in a study of the carbohydrate translocation of the Cuthbert raspberry concluded that sucrose serves essentially as a translocatory form and that reducing sugars are also mobile. CLEMENTS (**1**) found reducing sugars more abundant than sucrose in the petioles of sunflower, soybean, and potato. Furthermore, sucrose was at times absent from the petioles when translocation was occurring.

The mechanism responsible for transport in the phloem has been a subject for much conjecture. Reviews of this subject have recently been presented by CURTIS (**9**), MASON and PHILLIS (**22**) and CRAFTS (**7**). At present there are two widely differing schools of thought. CRAFTS (**8**) has divided them as follows: "(1) Those who attribute rapid diffusional movement of solute molecules to a specialized activity of the sieve tube protoplasm and (2) those who picture a flow of solution through perforate or permeable elements resulting from a gradient of hydrostatic pressure developed osmotically." Exudation of material from cut stems of cucurbits has been considered strong evidence for the latter. CRAFTS (**3, 4, 5, 6, 7, 8**) has presented this argument fully. MASON, MASKELL, and PHILLIS (**20**) have pointed out that whatever the pressure gradient in the intact stem may be, at the moment of cutting, it is opposed only by atmospheric pressure. Thus, the pressure gradient across the cut end is enormous. CRAFTS (**6, 7**) now recognizes that the flow produced by cutting is very largely caused by a sudden destruction

[1] Published with the permission of the Director, as Technical Paper no. 67, of the Hawaii Agricultural Experiment Station.

of a pressure equilibrium between xylem and phloem systems. That flow may be induced to continue by repeatedly removing thin slices of the stem, is taken to indicate establishment of a new equilibrium between xylem and phloem systems. He thinks that this equilibrium is analogous to one existing in the intact plant which causes a normal flow of solution. It might be pointed out, however, that the flow obtained by repeated cutting may be largely caused by the maintenance of a zero turgor pressure for the phloem at the cut end. There seems to be no evidence that a condition analogous to this occurs in the unsevered plant. According to calculations by MASON, MASKELL, and PHILLIS (**20**) the sieve plates alone would not offer too great a resistance to flow, if they are normally open. Yet microscopic observations show the pores to be filled with cytoplasm. Consequently, CRAFTS (**6, 7, 8**) maintains that the "pressure flow" proceeds through the phloem as a whole, walls and lumina as well. Upon cutting the cucurbit stem, however, the only internal evidence of any flow under pressure is slime-plug formation at the sieve plates. Does this not indicate that the exuded material comes from the sieve tube lumina and has been forced through the sieve plates?

The mass-flow hypotheses postulate a high turgor pressure gradient in the phloem. It is thought that removal of osmotically active materials from solution in regions of storage or utilization might maintain an osmotic gradient in the system. HUBER, *et al.* (**14**) find positive concentration gradients in exudate from a number of trees. CRAFTS (**4**) presents data showing gradients in the osmotic pressure of phloem exudate from cucurbits. It has not been positively demonstrated, however, that an osmotic gradient exists in the phloem or in the sieve tube system. CURTIS and SCOFIELD (**10**) found higher osmotic values in receiving organs than in supplying organs. This would appear not to substantiate the idea that an osmotic gradient is maintained by removal of osmotically active materials from solution in receiving organs.

MANGHAM (**17, 18**), appreciating the significance of plasmodesmata in affording continuity of protoplasm throughout the plant, considered that sugars moved on colloidal surfaces. CLEMENTS (**2**) has suggested ". . . that surface forces kept active by the respiration of living substances are at play. . . ." That respiration may play an important rôle is indicated by the apparent necessity of oxygen for transport (**21**). An hypothesis that movement proceeds along a diffusion pattern is presented by MASON and PHILLIS (**21, 22**). Evidence has been given that actual movement occurs at a rate of the order of 40,000 times greater than can be accounted for by simple diffusion through water (**19**). They suggest that ". . . in the sieve tube the cytoplasm as a whole is activated by metabolic energy and that it behaves as a liquid with diffusion constants enormously greater than those in water, . . ." (**22**).

Since vegetative growth as well as storage necessitates translocation of carbohydrate from leaves, it is apparent that the quantity and the direction of the major movement are dependent upon the state of metabolism of the plant. KRAUS and KRAYBILL (**15**) first demonstrated that vegetative and reproductive growth are conditioned by carbohydrate and nitrogen contents. Many chemical studies have followed dealing with relative carbohydrate and nitrogen contents of many plants under a great variety of conditions. From this wealth of material, it has developed that no trustworthy picture of the metabolic state of plants may be obtained solely from their carbohydrate and nitrogen contents; but when these are considered relative to type of growth, plant vigor, leaf color and environmental conditions, they assume a real significance. Furthermore, as NIGHTINGALE (**24**) has made clear, extremes of nitrogen deficiency on the one hand and of carbohydrate deficiency on the other have been repeatedly demonstrated to be associated with their respective types of plant response.

Plants in a growing vegetative condition move growth materials to meristems. For such plants organic nitrogen synthesis is the dominant phase of metabolism. Carbohydrates and their derivatives are here utilized rapidly in the formation of amides, amino acids, and proteins, and in respiration attending synthesis of organic nitrogen from nitrates. Clearly, in such vigorous plants, utilization and therefore translocation of carbohydrates is rapid. On the other hand, as plants attain a balanced fruiting condition, vegetative growth and the carbohydrate demand associated with it diminish; instead, carbohydrates and their derivatives are moved to fruits and are utilized in fruit growth and storage. Plants deficient in nitrogen cease fruit development as well as vegetative growth, and carbohydrate consumption and movement is much reduced.

The present investigation concerns cucurbit plants in three distinct metabolic categories: (1) young plants in a strongly vegetative condition, low in carbohydrate content; (2) older plants in a balanced fruiting condition, deficient neither in carbohydrates nor in nitrogen; (3) fruiting plants of the same age as (2) but deficient in nitrogen. Observations of conditions of growth and environment as well as chemical analyses of leaf tissue and phloem exudate were made with the view of determining: (1) the extent to which phloem exudate represents sieve tube contents; (2) the forces active in causing exudation; (3) some information relating to the mechanism involved in phloem transport.

Materials and methods

CULTURE

The Early Bush Scallop variety of *Cucurbita pepo* was used in these studies. The plants were grown in a greenhouse in water culture and sup-

plied with forced aeration. The composition of the nutrient solution was as follows: KH_2PO_4, 0.0050 M; $Ca(NO_3)_2$, 0.0100 M; $MgSO_4$, 0.0050 M; $MnSO_4$, 1.0 mg./l.; H_3BO_3, 0.5 mg./l.; $FeSO_4$, 5.0 mg./l.

Beginning with the second week, the solutions were changed weekly. Additional iron was added during the week as needed. The pH of the solution was maintained within the range of brom-cresol green (3.8–5.4) by addition of normal H_2SO_4.

Ninety seedlings were planted in thirty 2-gallon glazed crocks on January 14, 1939. On February 11, the series A collections were made from sixty of the most uniform plants. On February 18, thirty of the most vigorous plants were selected and placed in separate crocks. These were then divided into two treatment groups: in one group nitrate was applied as previously; in the other, nitrate was reduced to 0.0018 M concentration, and on February 26, excluded entirely.

In each case calcium nitrate was replaced by equimolar concentrations of calcium chloride to maintain the calcium content as well as the osmotic pressure. The series B collections were made on March 5.

A second study using this procedure was carried out during the summer of 1939. The results from this study are in general harmony with those from the winter study. For this reason, data from the plants grown in the summer will be reported only in a supplementary manner to present additional information and points of difference.

Methods of collecting

The plants used are characterized by short, stout stems and large leaves with long petioles. The petioles afforded the most convenient material for collection of exudate and were thus used exclusively in this study.

At the time of the series A collection, the plants had 5 to 6 fully expanded leaves. Two leaves were selected from each plant on the basis of petiole length, blade size, and color. Only one leaf from each plant was used in a given collection.

On the day previous to the series B collections, 5 plants from the plus-nitrogen group and 8 from the minus-nitrogen group were selected. Six mature vigorous leaves were selected from each plant. To limit photosynthesis to the blades, the petioles of the selected leaves were covered with black paper. A single sample consisted of material from two petioles from each of 5 plants. Samples were collected from the series A group at five times during the day; from the series B plus-nitrate group three times, and from the series B minus-nitrate group four times. The greenhouse atmosphere ranged between 71° F. and 91 per cent. relative humidity before sunrise to 83°–84° F. and 81 per cent. humidity at noon on each of the two days when collections were made.

The technique employed in collecting the exudate is that described by CRAFTS (**4**). Weighed vials (50 × 20 mm.) were used. Only that exudate which flowed in the first minute after cutting was collected. Collection in each case was made from the portion of the petiole remaining attached to the plant. Exudate was obtained from two portions of each petiole: the "upper petiole," one centimeter from the leaf blade; and the "lower petiole," five centimeters from the stem attachment. The exudate obtained from 10 to 14 "upper petiole" segments was combined and comprised one sample. The exudate obtained from the "lower petioles" comprised a separate sample. The time necessary for one collection varied from thirty minutes to an hour. The vials were tightly stoppered as soon as a collection was completed.

The samples for tissue analysis consisted of: (1) the "upper petiole," the segment extending from 2 to 14 cm. below the blade; (2) the "lower petiole," extending from the petiole base to 6 cm. above it; and (3) the blade. During the collection cut tissue was kept under a moist cloth.

Analytical methods

TISSUE

In the laboratory the plant tissue was quickly weighed and sliced. Fifteen- to thirty-gram fresh samples were killed in a volume of 95 per cent. boiling alcohol previously calculated to make the final concentration 75 per cent. In the case of the blades, additional 75 per cent. alcohol was added to cover the tissue. Extraction of soluble material was accomplished by decantation (**16**). Alcohol was removed from 100 to 200 ml. of the extract on a hot plate. Calcium carbonate was added to prevent acid hydrolysis of sucrose. The material was clarified with a slight excess of lead acetate and filtered. Excess lead acetate was precipitated with dry disodium phosphate. Aliquots of 25 ml. of cleared extract were made acid to methyl red with 10 per cent. acetic acid and treated with two drops of a fresh 0.5 per cent. solution of invertase scales. After 20 hours, reducing material present was determined as an estimate of total sugars. Total sugars and reducing sugars were estimated by the method of STILES, PETERSON, and FRED (**27**). Sucrose was obtained by difference. The nitrate present in the same extract was estimated by the phenoldisulphonic acid method (**25**). Total soluble material was estimated by drying aliquots of the alcohol extract at 65°–70° C. The residue from the alcohol extract was also dried at 65°–70° C. and weighed. Insoluble nitrogen was determined in duplicate weighed aliquots of the dried and powdered residue by the micro-Kjeldahl method of PREGL (**26**). Eight drops of 30 per cent. hydrogen peroxide were added at intervals during the digestion. Protein was estimated by multiplying

the milligrams of nitrogen obtained by 6.25. Qualitative tests for starch were made with an iodine solution.

Exudate

The fresh weight of the exudate was obtained by difference immediately after collection. The protein material was then precipitated by addition of 4 to 5 ml. of 95 per cent. alcohol. The material was dried at 65°–70° C. and weighed. Reducing material was extracted from the dried material by repeatedly washing with small amounts of boiling water and decanting through washed Whatman's no. 40 filter paper. The extract was then diluted to 25 ml. in each case. Duplicate 4-ml. aliquots were used for the determination of reducing material. To these, 1 ml. of 0.1 per cent. anhydrous dextrose solution was added, and total reducing material determined as for tissue extracts. To determine total sugars, 10 ml. of the extract was made acid to methyl red by addition of one to three drops of 10 per cent. acetic acid. One drop of a 0.5 per cent. fresh solution of invertase scales was added. The material was allowed to digest for 20 hours at room temperature. Reducing material was then estimated as before. Blank determinations were made on distilled water receiving the same reagents. The reducing power of 1 ml. of the 1 per cent. dextrose solutions was determined after addition to 4 ml. of distilled water. The reducing material in the extracts was then obtained by difference between the extract plus dextrose solution and the dextrose plus water solution. Sucrose was determined by difference between reducing material before and after inversion.

Qualitative tests with diphenylamine showed nitrate to be present in all extracts. Tests with phenoldisulphonic acid indicated a nitrate-nitrogen content of less than 0.05 mg. in all samples, which is less than 0.15 per cent. of the dry weight of the smallest sample.

In the summer study, 5-ml. aliquots of the extract were dried at 65° C. The soluble solids were estimated by weighing by difference. This dried material was then dissolved in concentrated sulphuric acid and the soluble nitrogen estimated by the micro-Kjeldahl method.

The residue remaining after extraction and the filter paper through which the extract was decanted were dissolved in concentrated sulphuric acid. Protein was then estimated as for the tissue. Blank determinations were made on comparable filter paper.

Experimentation

Growth of the plants

The first series of collections was made 27 days after the seedlings were placed in nutrient solution. The first 2 weeks of this period were characterized by a number of bright, warm days. In the third week, there was

scarcely a day when the sky was not overcast. This lack of sunlight continued until the day preceding the collection. The atmospheric conditions were reflected in the outward appearance of the plants. Thus, in the first 2 weeks the plants put forth a vigorous, balanced growth. At the beginning of the fourth week, the leaves were becoming darker green in color, and at the time of collection, their appearance suggested a type of growth characteristic of high nitrogen content or possibly carbohydrate deficiency. The data appear to confirm the latter as will be shown presently.

The series B collections were made 49 days after planting of the seedlings. The 22-day interval between the two collections was a period of sufficient sunlight to result in full and vigorous growth. By March 20, 2 weeks previous to the series B collection, flowers were abundant on all plants. At the time of these collections, fruits were developing rapidly on all plants, but were beginning to absciss from the minus-nitrate plants. The leaves of plus-nitrate plants were of a light green color, whereas those of the minus-nitrate plants were showing symptoms of their lack of nitrogen in their yellow green appearance.

Average lengths of petioles and blades are shown in table I.

TABLE I

LENGTH OF PETIOLES AND BLADES; AVERAGE OF 10 TO 15 LEAVES

Group	Length of petiole	Length of blade
	cm.	*cm.*
Series A	23	16
Series B + N	32	18
Series B − N	22	15

The average fresh weight per blade was 4.83 gm. for the young series A plants, 8.32 gm. for the plants of the series B plus-nitrate group, and 4.60 gm. for those of the series B minus-nitrate group.

From these data, it is seen that the maximum leaf expansion and petiole length is attained by the series B plus-nitrate group of plants. The leaves of the series A group are much smaller. This is due to the fact that, although the leaves were fully expanded at this stage, the plants were still very young. The minus-nitrate group shows a lack of maximum expansion caused directly by the lack of nitrogen during development.

In brief, the picture presented is: The series A plants were in a vegetative condition. The dark green leaves which were sampled were produced by young, vigorously growing plants low in a source of carbohydrate, under conditions where carbohydrate synthesis was limited, but exposed to conditions ideal for synthesis one day previous to sampling; the series B, plus-nitrate plants were older, were nutritionally balanced and at a fruiting stage

petiole tissue than in the exudate. In the minus-nitrogen plants, this difference was considerable.

These sugar concentrations, as well as the protein concentrations, demonstrate a highly local origin of the exudate. When the petiole is cut, the exudate can be seen to accumulate at the cut end of the phloem regions. The slime plugs that are formed in the sieve tubes occur only in the mature elements which have fully developed sieve plates (**4**). This is the only internal structural disturbance that is observed as a result of cutting. It is then likely that the solid material of the exudate consists largely of material from the mature sieve tubes.

Microscopic observations show that most of the cross-sectional area of the petioles is occupied by very large extra-vascular parenchymatous cells. Consequently, the chemical analyses of the petiole tissue, when expressed as percentage of the fresh weight, are to a large extent estimates of the composition of the extra-vascular parenchyma. The rather large differences between sugar concentration in the tissue and in the exudate as well as the differences in tissue concentration among the three groups of plants indicate that the concentration of sugars in the parenchyma must have little influence on that in the sieve tubes.

It is worthy of note that only rarely does the total sugar concentration exceed 0.5 per cent. of the fresh weight of the exudate. In one case exudate was collected from the cut stem of a vigorous plant and its total sugar content was found to be 0.20 per cent. of the fresh weight. In a collection of exudate from *Cucurbita maxima,* the total sugar concentration was 5.4 mg./ml. (**23**). None of these concentrations even approaches the 9 per cent. solution supposed by CRAFTS (**3**) for calculating rates of solute movement into a pumpkin. It seems highly improbable, therefore, that accumulation of organic material in the pumpkin is a result of a mass flow of sugar solution.

In table VIII is presented the fresh weight of exudate per petiole and the dry weight as percentage of fresh weight for the three groups. It is seen here that the flow was greatest from the minus-nitrate petioles and least from the low-carbohydrate petioles of series A. This is the reverse order if dependent upon sugar concentration of the exudate. Indeed, the amount of flow is directly correlated with the sugar content of the tissue instead. Furthermore, the greatest flow occurred from the minus-nitrate plants where translocation was much reduced. It is recalled that abscission of fruits was occurring in this group. The amount of flow from petioles is again of lowest magnitude in the vegetative group where the mature leaves were supplying carbohydrates to the rapidly growing meristems. The plus-nitrate petioles of series B were considerably larger than the minus-nitrate petioles, yet flow of exudate from the former was smaller. On these

bases it appears that no relationship between flow of exudate and translocation exists.

TABLE VIII

AVERAGE FRESH WEIGHT OF EXUDATE PER PETIOLE AND DRY WEIGHT AS PERCENTAGE OF FRESH WEIGHT.

MILLIGRAMS OBTAINED IN ONE MINUTE AFTER CUTTING

SERIES	NUMBER OF PETIOLES AVERAGED	FRESH WEIGHT PER PETIOLE		DRY WEIGHT AS PERCENTAGE OF FRESH WEIGHT	
		UPPER PETIOLE	LOWER PETIOLE	UPPER PETIOLE	LOWER PETIOLE
		mg.	*mg.*	%	%
A	70	10.7	20.0	8.71	9.00
B + N	30	24.4	32.8	8.34	8.79
B − N	35	28.6	41.8	8.57	8.82

The data presented here afford little evidence that movement of carbohydrates occurs as a result of a mass flow of solution through the sieve tubes. Moreover, the protein content of the exudate is indicative of a dense protoplasm in the sieve tubes. Associated with this protoplasm may be a high metabolism as shown by an apparent accumulation of sugar against a gradient in the low carbohydrate plants. A cellular organization having these characteristics would surely play anything but a passive rôle whatever its function.

HAMNER (**13**) has presented evidence that the reduction of nitrate in plants is associated with a high respiration. MOOSE (**23**) found pH values of 8.0 and 8.1 in exudate of *Cucurbita maxima*. A pH value between 6.8 and 7.4 was obtained with range indicators on one occasion in the course of the present work. Such a neutral or slightly alkaline reaction is proper for nitrate reduction as shown by ECKERSON (**11**). This observation together with the low nitrate content may indicate that reduction of nitrate occurs actively in the sieve tubes. If this is the case, it lends further support to the concept of a highly active phloem.

It is now necessary to consider: (1) the extent to which translocation from the leaves of the various groups has occurred; and (2) the factors which contribute to a flow of exudate from the phloem when the petiole is cut.

MOVEMENT OF SUGARS

The total sugars of the tissue of blade and petiole segments are shown in table V. In series A the sugar content of the blade is at its low level at 8:00 A.M. It rises from 8:00 A.M. to a maximum at 12:00 M., diminishing rapidly thereafter. The petiole segments show a similar trend. These petioles were not covered, so photosynthesis was normal. These trends in

the blades represent little more than the amount of sugar being formed relative to the amount being used and translocated. In the petiole segments the amount present at any time is dependent upon the additional factor of the amount being moved in from the blades. For a period in the morning, the rate of sugar formation in the blades was exceeding the sum of the rates of removal and utilization. Throughout the latter part of the day, the rate of removal plus the increased respiration, owing to higher temperature, was exceeding synthesis. These plants were rapidly growing, necessitating rapid movement of growth materials, yet at no time was there a significant concentration gradient from blade to upper petiole or from upper to lower petiole.

In the plus-nitrate plants of series B, there is a marked difference in concentration between the blades and petioles. Between 8:00 A.M. and 12:00 M. a greater rate of formation than removal is again shown in the blades. The upper petiole shows loss of sugar at 12:00 M. possibly because the petioles were covered with black paper preventing synthesis, and speeding up respiration in this segment. The lower petiole shows no significant change during this period; supply and removal have been balanced. In the minus-nitrate plants, a still greater difference in concentration exists between blades and petioles. These blades show a greater rate of synthesis than removal throughout the whole day. There is a pronounced increase in sugar in both petiole segments even though these petioles were covered. A greater rate of movement into than of removal out of the segments accounts for this accumulation. This low rate of removal is associated with a sluggish metabolism and is in harmony with the observations of stunted growth and falling of fruits.

Factors causing exudation

In series A there was a pronounced decrease in the amount of exudate which flowed from a cut petiole as the day progressed. That this is associated with a water loss from the tissue is indicated (fig. 1). The fresh weights of the segments sampled are expressed as the percentage of the maximum. It should be remembered that the individual values are subject to sampling errors because of the relatively small number of segments used. It is also not known that the actual maximum and minimum points were obtained. Yet, the maxima and minima which were found for fresh weight of tissue closely correspond to maximal and minimal flow of exudate, respectively. A direct relationship between water content of tissue and exudation is indicated.

The flow of exudate from the upper petiole was curtailed more by water loss than was that from the lower petiole. The loss of water, however, from the tissue of the upper petiole was less; a smaller degree of water loss in the

upper petiole probably affected flow of exudate more. This may be explained by a higher ratio of vascular tissue to parenchyma in the upper petiole. An approximate measure of this ratio is the insoluble material of the tissue expressed as percentage of fresh weight. The extra-vascular parenchyma consists of very large thin-walled cells contributing very little to the insoluble solid material. The vascular material, on the other hand, contributes largely to the insoluble material of the tissue due to secondary

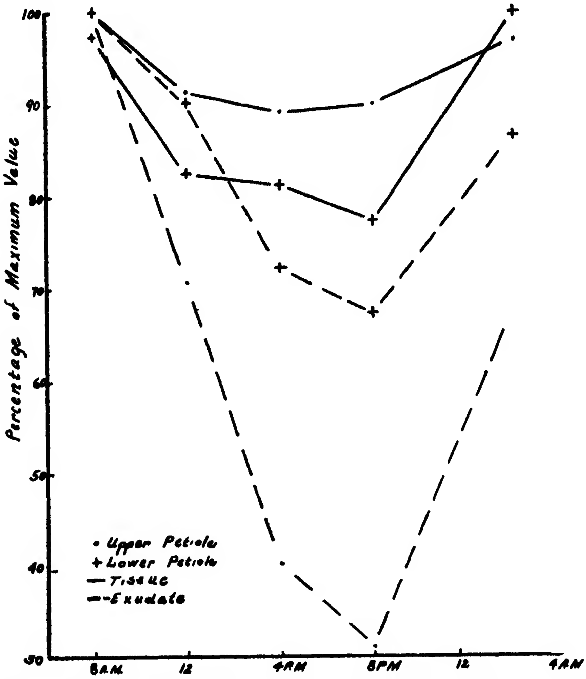

FIG. 1. Fresh weight of the petiole tissue and of the exudate as percentage of the maximum value. Series A.

thickening of the xylem elements and highly proteinaceous phloem. Table II shows a higher insoluble solid content in the upper petiole for this group.

When much collenchyma exists in the upper petiole, it could contribute to this difference; to avoid this possible error, a comparison of only the protein material may be made. That the protein material is to a large extent restricted to the phloem has already been shown. Table IV shows that this, too, is higher in the upper segment than in the lower.

It thus appears that the water loss which may take place from a segment of the petiole is directly related to the amount of extra-vascular parenchyma. From this, it follows that the amount of phloem exudation is dependent in part upon the water content of the extra-vascular parenchyma. Of course, it is recognized that water loss from the tissue of the blades as well as the

petioles is associated with a greater negative tension in the xylem as the day proceeds. This is undoubtedly effective in decreasing exudation.

For series B, the fresh weight of the tissue of the petioles is plotted in figure 2 as percentage of the maximum. There appears to be no significant change in water content of the tissue of the plus-nitrate plants between 8:00 A.M. and 12:00 M. In the minus-nitrate plants, there appears to be a significant loss of water from the tissues during this period. In neither upper nor lower petiole is the water loss as severe or as extended as it was in series A. The minus-nitrate petioles had regained their turgor at 6:00 P.M., while it will be recalled that the turgor of the petioles of the series A group was not regained even at 8:00 P.M.

Between 8:00 A.M. and 12:00 M. there was a very marked increase in exudation from the plus-nitrate petioles. Though not so marked, a similar response is seen in the minus-nitrate plants. This is in sharp contrast to the result for the same time of day in series A. There was very little difference in the two days as far as temperature and humidity are concerned. There was no appreciable air movement on either day. The water loss from the blades of all groups of plants was marked during these intervals (*i.e.*, as water per blade: 0.34 gm. in series A; 0.74 gm. in the series B plus-nitrate group; and 0.40 gm. in the series B minus-nitrate group). Water loss due to transpiration was thus proceeding at a more rapid rate than was uptake by the blades. There is, then, every indication that a high negative tension existed in the xylem in all groups.

It is evident that the explanation of exudation on cutting lies in a consideration of the pressure relations of the tissues of the intact plant and in how these relations will be affected by cutting. This involves the functional pressure[2] of the cells of each tissue involved (that is, the pressure operative in producing a movement of water into or from them). This is the net effect dependent upon the osmotic and turgor pressures.[3]

In the petioles of *Cucurbita pepo,* the extra-vascular parenchyma consists of very large, thin-walled cells. The water content of these cells is very high and will be affected to a considerable degree by functional pressures to and from them. They are cells capable of attaining considerable turgidity when conditions are favorable for movement of water into them; on the other hand, they can withstand considerable loss of water when the functional pressure is out of them. The xylem, in contrast to the parenchyma, is characterized by elements with secondary thickening capable of resisting col-

[2] This is the pressure commonly given a number of names such as suction pressure, suction tension, net pressure, etc.

[3] Osmotic pressure is here considered the vector tending to move water into a cell due to the effect of the solutes of the cell sap in reducing its vapor tension. By turgor pressure is meant the vector resisting further water intake due to confinement within the cell.

lapse under conditions of deficit. CRAFTS (3) has shown that the phloem cells have thick, highly hydrated walls. An active sieve tube system would seem capable of developing a considerable turgor pressure.

The total amount of water-soluble solids in exudate from petioles of plants grown during the summer of 1939 was about 70 per cent. of the total dry weight. The exudate contains an approximate 6 per cent. (ash shown in table VIII, the dry weight ranges between 8.3 and 9.0 per cent. of the fresh weight) solution of water soluble material of which not more than 0.5 per cent. is sugar. When this is compared to the 2.3–3.4 per cent. solution of the petiole tissue (table II), it would appear that the exudate in each case has a greater osmotic pressure than has the tissue. The osmotic pressure of the exudate, however, is attributable mostly to soluble material other than sugar.

Fully aware of these tissue relations, we are in a position to consider what takes place when the petiole is cut. Let us take first an early morning collection from the low carbohydrate plants of series A. We know that the rate of transpiration from the leaves did not greatly exceed the rate of uptake of water because the water content of the blades was at a maximum at this time. Consequently, there was little if any negative tension in the xylem, and the parenchyma and sieve tubes were turgid. Cutting the petiole resulted in a release of the turgor pressure of the sieve tube system causing a functional pressure into it from the xylem and parenchyma. The exudation which occurred was a result of this water flushing the sieve tube contents from the cut petiole.

As the day progressed and the temperature rose, the rate of transpiration exceeded water uptake resulting in an increasing negative tension in the xylem elements. This was associated with a water loss from the extra-vascular parenchyma of the petioles, reducing their turgor pressure and increasing their osmotic pressure. It is likely that the turgor of the phloem was similarly reduced. The phloem, however, because of its higher osmotic pressure and the thicker cell walls, would not be expected to lose as much water as the parenchyma. Cutting the petiole under these conditions would instantly release the negative tension in the xylem causing a now unbalanced functional pressure to operate from the xylem into the parenchyma and phloem. The already reduced turgor pressure of the phloem is released by the cut as before, but the much reduced turgor pressure of the parenchyma this time augments the functional pressure from the xylem into it. As a result exudation from the phloem does occur, but not to the degree it did in the early morning, since much of the xylem water now moves into the parenchyma rather than wholly into the phloem.

In series B both the plus- and minus-nitrate plants have a much higher sugar content in their petiole tissues than have those of series A. That this

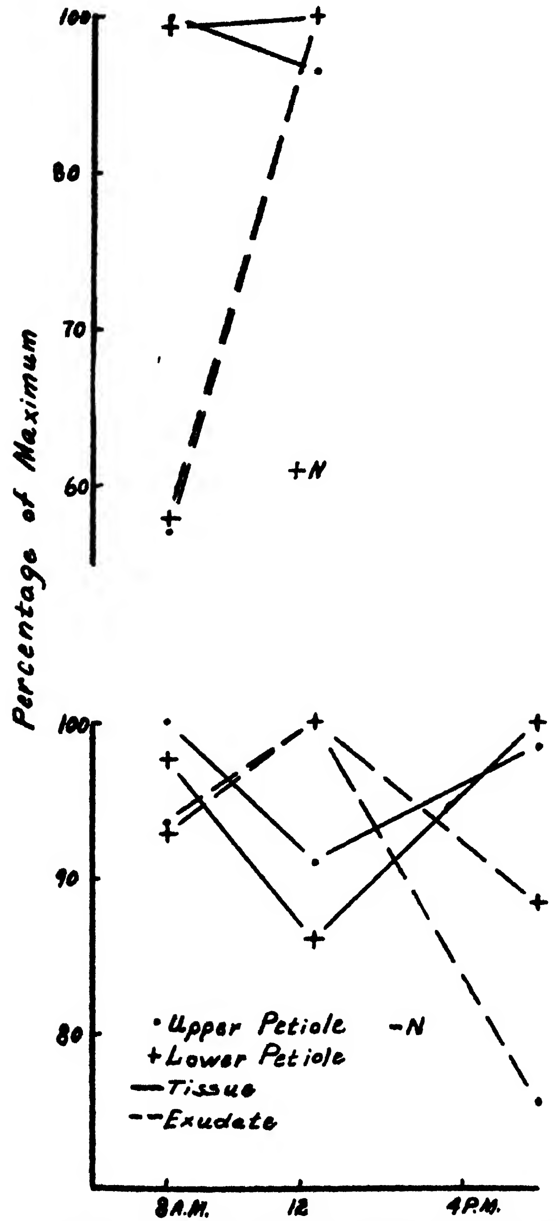

FIG. 2. Fresh weight of the petiole tissue and of the exudate as percentage of the maximum value. Series B.

higher total sugar content contributed largely to a higher total soluble solid content is shown in table VI. There is little difference in the nitrate content of the plus-nitrate plants in the two series. The undetermined soluble material represents nearly half of the total soluble solids in each case. This fraction as well as the total sugars is higher in series B. What proportion of this material is represented by pigments and other substances which would contribute little to the osmotic pressure is not known. It is possible that a considerable proportion of it represents inorganic ions which are osmotically active.

We may now consider the plus-nitrate plants of series B. The parenchyma of these, we have reason to believe, has the highest osmotic concentration of any of the groups. Analysis has shown the sugar and nitrate to be much lower in the exudate than in the tissue as a whole. It has been noted that exudation was much greater at 12 : 00 M. than at 8 : 00 A.M. Since there is little difference in the quantity of solutes present at the two times, the principal variable appears to be the degree of tension in the xylem system and the pressure differences imposed by it upon the parenchyma and sieve tubes.

In the morning, when tension in the xylem is positive or only slightly negative, water may be taken from it readily by the osmotically active cells until the movement into them is checked by their own turgor pressure. A cut at this time will release the turgor pressure of the phloem allowing water to enter it from the xylem causing exudation. Imposing a high negative tension on the xylem system will reduce the turgor of the phloem and parenchyma as in series A. In series B, however, the higher osmotic pressure of the parenchyma will resist water loss, resulting in less turgor reduction than occurred in series A. Cutting will now result in release of the negative xylem tension as well as the remaining turgor of the phloem causing a functional pressure to the greatest extent into the phloem and giving more exudation than occurred before.

In this manner a higher negative tension in the xylem might enhance exudation when a high concentration of osmotically active solutes exists in the parenchyma whereas an increasing negative tension in the xylem may reduce exudation where the osmotic concentration in the parenchyma is low. The occurrence of exudation has been explained by creation of a functional pressure into the sieve tubes when the cut is made. It would follow that in each case exudation is accompanied by some dilution. Consequently, none of the analytical values for the exudate represents the actual content of the sieve tube. If the actual composition of the phloem were the same throughout the day, the maximum concentration of material observed in the exudate would be the closest approach to that occurring in the sieve tube.

Summary

1. Plants of *Cucurbita pepo* in the following physiological conditions were used in these studies: (1) young plants in a strongly vegetative condition, low in carbohydrates; (2) older plants in a fruiting condition, deficient neither in carbohydrates nor in nitrogen; (3) plants of the same age as (2) but deficient in nitrogen.

2. At certain intervals of the day, collections were made of the exudate which flowed from the cut portion of the petiole during the first minute after cutting. Collections of tissue from the leaf blades and petioles were also made.

3. Reducing sugars, sucrose, protein, soluble nitrogen, and nitrate in tissue and in exudate were quantitatively determined.

4. That the plants represented three distinct metabolic states is shown by observations of type of growth: leaf color, length, and weight as well as protein, sugar, and total solid content.

5. Translocation is demonstrated in the minus-nitrate group by an accumulation of sugars in the petioles which were covered with black paper. In the other two groups sugars did not accumulate in the petioles, and because of the growth behavior of the plants translocation was known to be rapid.

6. The sieve tubes seem to be the principal source of the solid material of the exudate. The exudate material appears to be in all cases diluted with water from the extra-sieve tube tissue.

7. Sugar concentrations observed in the exudate are lower than any of those which have been used as a basis for calculating rates of flow of solution in the phloem.

8. An active sieve tube protoplasm is indicated by a high protein and soluble nitrogen content in the exudate as well as an apparent accumulation of sugars against a gradient in the young plants.

9. The sugar content of the exudate was not related to sugar content of the associated tissue, but appeared to be correlated with the metabolic level of the plants.

10. A consideration of the effect of cutting the petiole on tissue-pressure equilibriums existing in the intact plant lends a plausible explanation for the occurrence of exudation as well as differences in exudation.

11. The amount of exudate flowing from a cut petiole is not dependent upon translocation or upon the sugar concentration of the exudate. The data show little evidence of a "pressure flow" mechanism in the phloem.

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THE EFFECT OF SALT CONCENTRATION UPON THE METABOLISM OF POTATO DISCS AND THE CONTRASTED EFFECT OF POTASSIUM AND CALCIUM SALTS WHICH HAVE A COMMON ION[1]

F. C. STEWARD AND C. PRESTON

(WITH THREE FIGURES)

Introduction

The metabolic processes which have been observed in potato discs under the conditions conducive to salt accumulation have been described (**20**) and a balance sheet of metabolites prepared (**21**). This work revealed certain relationships between the nature of the salt supplied and its effects upon the respiration and nitrogen metabolism of the cells. These results gave a new outlook upon the problem of salt absorption and, therefore, the relationship of the metabolism of potato discs to salts was intensively and systematically investigated.

The purpose of the further investigation was to identify the specific effects on metabolism of the most important inorganic anions and cations and, therefore, to provide the essential biochemical background for a fuller understanding of salt absorption. Of necessity the work was confined to cells derived from one plant and organ—the potato tuber. Broad generalizations must ultimately seek their confirmation in the behavior of other cells and tissues. No apology need be made, however, for this first restricted attention to one type of cell which was investigated under rigorously controlled conditions.

The scope of the entire work may be indicated because it demands that the results shall be described in three parts—subsections which yet represent integral parts of the whole problem.

1. The first part deals with those effects on metabolism which are caused by neutral salts. The experiments described show the effects of the concentration of different potassium and calcium salts in the external solution and the effects due to the various anions and cations, respectively, stand revealed when experiments which involved the use of bromides, chlorides, nitrates, and sulphates of potassium and calcium are compared. Wherever possible concurrent experiments employed salts with a common anion and the data not only reveal the principal effects attributable to each ion but also the interaction of the effects of anions and cations.

2. The second part deals with the more difficult problems raised by those salts which not only affect the external reaction of the solution, but which

[1] This is the third of a series of papers on the biochemistry of salt absorption by plants. The authors are indebted to Prof. D. R. HOAGLAND for proof reading this paper.

TABLE VII

EFFECT OF EXTERNAL SALT CONCENTRATION ON THE SOLUBLE NITROGEN FRACTIONS OF POTATO DISCS*

SAMPLE	SOLUTION NO.	SALT CONCENTRATION	EXPERIMENT 1	TOTAL SOLUBLE NITROGEN	AMINO NITROGEN	HEAT STABLE AMIDE	HEAT LABILE AMIDE	SAMPLE	SOLUTION NO.	SALT CONCENTRATION	EXPERIMENT 2	TOTAL SOLUBLE NITROGEN	AMINO NITROGEN	HEAT STABLE AMIDE	HEAT LABILE AMIDE
				mg.	*mg.*	*mg.*	*mg.*					*mg.*	*mg.*	*mg.*	*mg.*
Initial			KBr	1.26	1.02	0.194	0.150	Initial			KCl	1.38	1.09	0.208	0.144
Final	1	0.00075	series	0.97	0.71	0.094	0.229	Final	1	0.00075	series	1.15	0.90	0.134	0.189
"	2	0.015		0.67	0.42	0.088	0.148	"	2	0.015		0.94	0.69	0.179	0.117
"	3	0.050		0.67	0.44	0.088	0.146	"	3	0.050		0.87	0.61	0.198	0.120
"	4	0.075		0.65	0.46	0.084	0.142	"	4	0.075		0.85	0.57	0.162	0.122
Initial			$CaBr_2$	1.32	1.06	0.188	0.158	Initial			$CaCl_2$	1.37	1.06	0.205	0.147
Final	1	0.00075	series	0.93	0.70	0.034	0.226	Final	1	0.00075	series	1.19	0.92	0.164	0.180
"	2	0.015		1.23	0.81		0.228	"	2	0.015		1.24	0.90	0.224	0.180
"	3	0.050		1.28	1.00	0.051	0.230	"	3	0.050		1.34	0.95	0.261	0.185
"	4	0.075		1.38	1.06	0.096	0.228	"	4	0.075		1.37	1.04	0.227	0.178

* All units in milligrams per gram initial weight.

The outstanding conclusion from these data is clear. All of the salt treatments (increasing concentration of external potassium salts) which increase respiration (table II, fig. 1) also increase the protein synthesis. This occurs, to some degree, at the expense of the soluble nitrogen reserves even in discs exposed to dilute salt solution or distilled water (**20**). Although time (not an absolutely constant factor throughout all of the experiments) is also involved, it is clear that the anion (NO_3) caused the greatest protein synthesis. It also accentuated the effect of potassium on respiration. Again the anion (SO_4) had but little effect on respiration; it also provided the least stimulus to protein synthesis when present as the potassium salt.

Conversely the salt treatments (increased concentration of calcium salts) which decrease respiration, also tend to suppress the synthesis of protein from the storage reserve of soluble nitrogen compounds. In the nitrate series this result apparently does not hold. Not until the nitrogen fractions are considered in greater detail can this anomaly be removed. It can be stated here, however, that the normal synthesis of protein in potato discs utilizes nitrogen mainly derived from amino compounds and *it is only when the nitrogen is derived from this source that the close parallelism between synthesis and respiration is obtained.* It has been indicated in an earlier work (**19**) that calcium tends to divert synthesis from the amino compounds to the other fractions of the soluble nitrogen. Furthermore, in the presence of nitrates, especially calcium nitrate, synthesis *may proceed directly from inorganic sources independent of nitrogen drawn from organic amino compounds.* The fact that the usual relation between synthesis and respiration apparently breaks down in the calcium nitrate series can therefore be explained.

Prior to the more detailed analysis of the nitrogen fractions, the interrelations of the effects of salt concentration on the processes thus far considered are summarized in figure 2 which is constructed from the data of tables II, IV, and VI. Respiration and sugar content are definitely affected in converse manner by salt concentration; compare the general trend, shown by the histograms, through the potassium series 4 to 1 and the calcium series 1 to 4. It is equally clear that the trend of protein synthesis (apart from the calcium nitrate series referred to above) is similar to that for respiration. In other words, figure 2 expresses the following in graphical form: *respiration and protein synthesis of potato discs are closely linked whereas there is no causal connection between respiration and sugar content* (as affected by external salt concentration).

Effects of salt concentration on the soluble nitrogen fractions

The detailed effects of salt concentration on the nitrogen metabolism of potato discs can be discerned from tables VII and VIII in which the data

are expressed in absolute units; and also from figure 3, in which the various fractions have been calculated relative to the initial total nitrogen content of the washed discs to which the value 100 was assigned for each experiment.

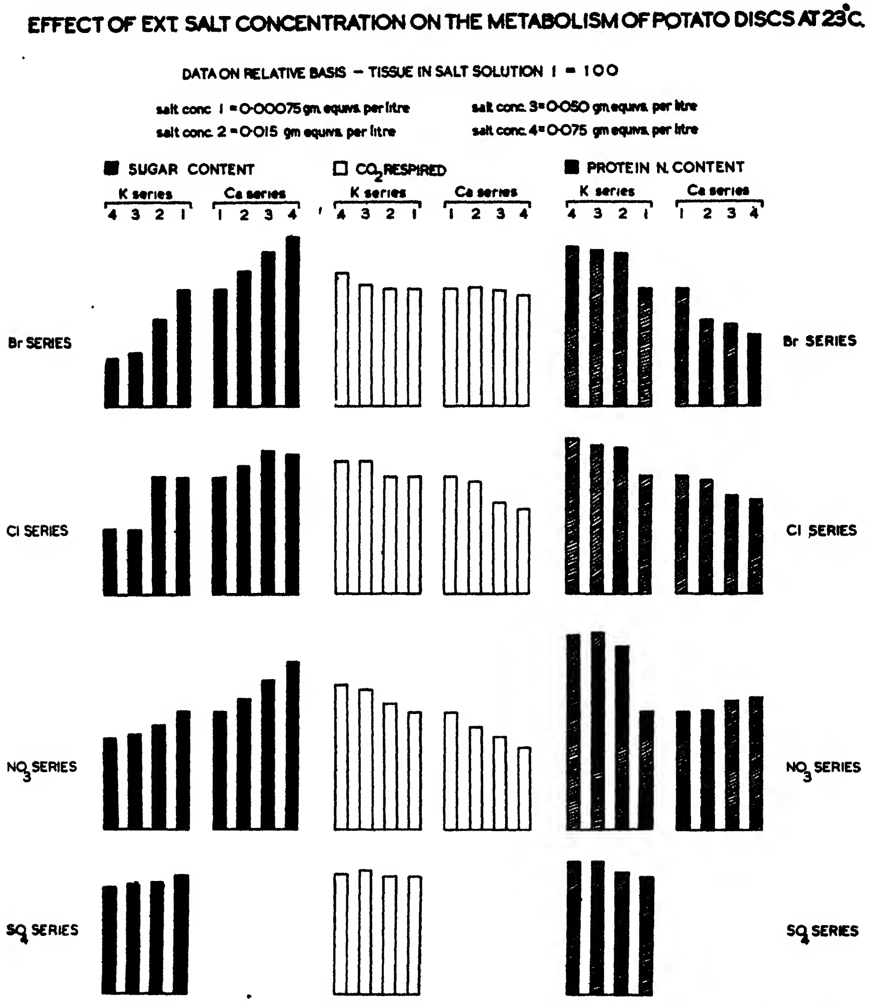

FIG. 2. Effect of external salt concentration on the metabolism of potato discs at 23° C.

From figure 3 the following conclusions, some of them already evident in the earlier data, can be verified.

(a). Protein synthesis (protein N represented by histograms below the line) occurs at the expense of the soluble nitrogen fractions (soluble N represented by histograms above the line) in potato discs exposed to dilute aerated salt solutions (see no. 1 of each series) at 23° C.

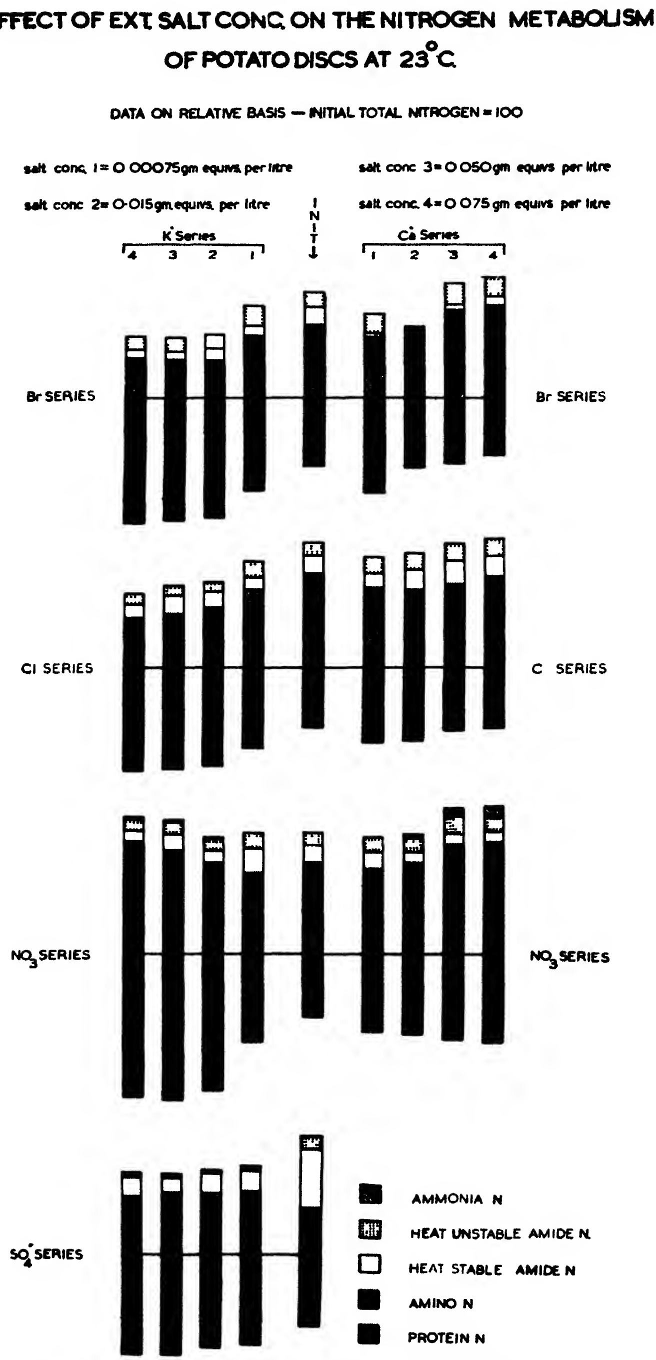

FIG. 3. Effect of external salt concentration on the nitrogen metabolism of potatoes at 23° C.

(b). In greater concentrations of potassium salts (chlorides and bromides) protein synthesis is increased. In solutions of sulphates the effect of potassium salt concentration on synthesis is slight.

(c). In solutions of nitrates the total nitrogen content is increased; more so in potassium than calcium salts of the same equivalent concentration. When nitrogen is absorbed from potassium nitrate, protein synthesis accounts for a large proportion of the *added* nitrogen (80 per cent. in solution no. 4) whereas in the calcium series the protein synthesized represents a much smaller proportion of the total nitrogen absorbed (55.6 per cent. in solution no. 4). In other words, although there is a greater total synthesis of protein in the stronger calcium nitrate than in the weaker solutions, *the effect of calcium as a depressant of synthesis is still apparent.*

TABLE VIII

EFFECTS OF EXTERNAL SALT CONCENTRATION ON THE SOLUBLE NITROGEN FRACTIONS OF POTATO DISCS*

Sample	Solution no.	Salt concentration	Experiment 3	Total soluble N	Amino N	Heat stable amide	Heat labile amide	Ammonia N
				mg.	*mg.*	*mg.*	*mg.*	*mg.*
Initial			KNO_3	1.33	1.03	0.192	0.150	0.002
Final	1	0.00075	series	1.31	0.93	0.243	0.166	0.002
"	2	0.015		1.31	1.05	0.126	0.144	0.021
"	3	0.050		1.48	1.19	0.175	0.151	0.037
"	4	0.075		1.50	1.29	0.119	0.149	0.041
Initial			$Ca(NO_3)_2$	1.36	1.06	0.189	0.147	0.002
Final	1	0.00075	series	1.26	0.99	0.195	0.153	0.002
"	2	0.015		1.24	1.06	0.120	0.202	0.050
"	3	0.050		1.66	1.29	0.124	0.278	0.100
"	4	0.075		1.60	1.31	0.120	0.286	0.104
Initial			K_2SO_4	1.10	0.51	0.605	0.141	
Final	1	0.00075	series	0.93	0.68	0.216	0.043	
"	2	0.015		0.87	0.66	0.199	0.033	
"	3	0.050		0.86	0.66	0.151	0.040	
"	4	0.075		0.80	0.62	0.201	0.041	

* All units in milligrams per gram initial weight.

(d). Amino nitrogen (amino acid + asparagine amino nitrogen) constitutes the largest soluble nitrogen fraction in all the tissue samples examined with the exception of the initial tissue used for the sulphate series. This contained much more amide nitrogen than the others.[6] Since this feature

[6] Some evidence obtained with potato tubers stored at low temperature (2° C.) shows that the relative accumulation of amide can be brought about by this treatment. In this extreme condition the high sugar concentration tends to produce a high rate of respiration which is different in its relations to nitrogen compounds from that observed in tissue drawn from normal tubers. The sulphate series showed a respiratory rate somewhat in excess of the normal value. There is every reason to believe that this tissue was drawn

disappeared under the experimental treatments it does not affect the comparison of the effects due to salt concentration *per se.*

(e). In the two bromide series the gain of protein in tissue exposed to the dilute solutions (no. 1) was almost balanced by the loss of amino-nitrogen (KBr loss of amino-N = 110 per cent. of gain of protein-N; $CaBr_2$ loss of amino-nitrogen = 103 per cent. of gain of protein-N) and in the dilute solution (no. 1) of the chloride series the gain of protein was also largely accounted for by loss of amino-nitrogen (79.2 per cent. for KCl and 73 per cent. for $CaCl_2$). As a result of increased potassium salt concentration (no. 4) the additional protein synthesized amounted to 0.37 (KBr series) and 0.29 (KCl series) mg. per gm. initial fresh weight and of this 0.25 and 0.33 mg. respectively could have been derived from the amino fraction. In the calcium bromide series the strong salt (no. 4) suppressed the synthesis of 0.43 mg. of protein nitrogen and of this 0.36 mg. (83.8 per cent.) remained as amino-nitrogen and in the calcium chloride series the synthesis of 0.17 mg. of protein-nitrogen was suppressed by the strong salt solution (no. 4) and 0.12 mg. of amino-nitrogen (70.7 per cent.) was conserved.

Clearly, then, the principal effect of the salt is upon the utilization of amino-nitrogen, the bulk of which is amino-acid amino nitrogen, in protein synthesis.

(f). With respect to amide the position is more complicated owing to the presence of amides of different degrees of stability—heat stable amides (asparagine) and heat labile amides (comparable to glutamine). In the dilute salt solutions (no. 1) all of the series (KBr, $CaBr_2$, KCl, $CaCl_2$) show an increase in the heat labile, easily hydrolyzable fraction which could account for a large part of the concomitant decrease in the stable amide fraction (KBr, 79 per cent.; $CaBr_2$, 44 per cent.; KCl, 61 per cent.; $CaCl_2$, 56.2 per cent). Increased concentrations of calcium salts, however, suppressed somewhat the disappearance of the amide fraction without affecting the accumulation of unstable amide appreciably. Also the increase in the concentration of potassium salts (difference nos. 1 and 4) decreases markedly the concentration of the unstable amide without a similar effect upon the reserve of stable amides.

It seems, therefore, that the heat labile, easily hydrolyzable amide fraction *does not arise directly from the more stable amide;* it accrues from ammonia which could be released mainly from amino compounds by oxidative deamination [see also (**20**)] and also in part by hydrolysis of the more stable amides. As previously noted (**20**) the unstable amide therefore appears as a reactive intermediary in the formation of protein and its final

from a stock which was in some degree affected by exposure to temperatures in storage lower than normal. There is no reason to suppose, however, that this feature determined the relationship to salt concentration which is here described.

concentration is *decreased by those salt treatments (K series) which greatly accentuate synthesis.*[7]

Certain parallelisms between the behavior of the unstable amide fraction and sugar will be apparent. The experimental conditions which cause a recrudescence of vital activity also stimulate starch hydrolysis, an increase in the concentration of sugar (culture no. 1: KBr, $CaBr_2$, KCl, $CaCl_2$ series), and a relatively high concentration of unstable, easily hydrolyzable amide.[8] Oxygen and temperature are no doubt the chief causal agents but the salt treatments (increased potassium salt concentration) which still further stimulate respiration and synthesis decrease both the sugar residue and likewise the accumulated unstable amide. The converse is not true. The salt treatment (increased concentration of the halides of calcium) which depresses respiration and protein synthesis conserves the sugar residue but the concentration of the unstable amide remains unaltered.

TABLE IX

EFFECT OF EXTERNAL SALT CONCENTRATION ON EASILY HYDROLYZABLE AMIDE CONTENT OF POTATO DISCS

SOLUTION NO.	SALT CONCENTRATION*	EXPERIMENT 1	RELATIVE CONTENT OF LABILE AMIDE	EXPERIMENT 2	RELATIVE CONTENT OF LABILE AMIDE
1	0.00075	KBr series	100.0	KCl series	100.0
2	0.015		64.6		62.0
3	0.050		63.8		63.5
4	0.075		62.0		64.5
1	0.00075	$CaBr_2$ series	100.0	$CaCl_2$ series	100.0
2	0.015		99.2		100.0
3	0.050		102.0		103.0
4	0.075		99.2		99.0

* Concentrations in gram equivalents per liter.

These points are shown in table IX in which the effect of salt concentration on the heat labile amide is shown by data calculated on a relative basis (unstable amide content in tissue of culture no. 1 for each series = 100).

[7] It is more usual to regard asparagine and glutamine as by-products of protein breakdown and somewhat off the main route of synthesis of protein from ammonia [see CHIBNALL (2, pp. 109 and 194)]—a synthesis usually supposed to reconstitute amino acids prior to protein formation. The more attractive view that the protoplast produces protein from sugar and ammonia without forming amino acids as intermediaries is admittedly devoid of direct evidence. The relations which CHIBNALL envisages between glutamine production and respiration concern the relation of glutamine to the cycle of metabolism of organic acids rather than protein synthesis.

[8] The sulphate series was done with tissue drawn from a separate stock which had an unusually high amide content in the initial washed discs. This is the only case of its kind observed in a great many such analyses and the difference between the initial and final tissue of this series is not used as the basis of any conclusion.

(g). When the cations are supplied as nitrates two factors require consideration. First, as illustrated by the data on respiration, the nitrate ion enhances the specific effects due to the cations; and, secondly, this anion can and does contribute to the soluble organic nitrogen fractions. After treatment in strong potassium nitrate the observed increase in the ammonia content of potato discs is slight. The nitrate entering from potassium nitrate forms chiefly amino nitrogen and protein and the metabolism, although much stimulated by potassium, does not cause a net loss of the amino fraction. In presence of relatively strong calcium salts the amino fraction is almost identical with that of the corresponding potassium cultures but the outstanding fact is that the other nitrogen absorbed by the tissue forms much less protein and remains much more in the form of ammonia and amide than in the corresponding potassium culture.

External potassium concentration, therefore, increases synthesis of protein from amino acid and in this the effects of potassium and oxygen are similar—the former accentuates the effect of the latter. Ammonia is not present appreciably in the tissue treated with potassium halides (and only slightly in potassium nitrate solutions) and the residual concentration of unstable amides is reduced by the greater synthesis which is stimulated by potassium. It follows that the effects of potassium salt concentration are not confined to the initial stages of the utilization of amino acids but extend to most, if not all of the reactions involved; especially those in which unstable intermediates formed from sugar and ammonia are probably concerned. Calcium salts have the converse effect on protein synthesis; they depress the use of amino-acids, conserve the heat stable amide, and (in calcium nitrate cultures) cause ammonia to accumulate appreciably. The concentration of intermediates (heat labile amides) remains unaffected by high calcium concentration at the high values (no. 1) which are attained when a low rate of protein synthesis does not consume these faster than they are formed. Inasmuch as calcium, unlike the effect of potassium, fosters the storage and preferential use of ammonia and amide in the tissue, *it diverts protein synthesis from the amino acids and encourages it to proceed* without that oxidative deamination of amino acids; the latter is now believed to form the most probable link between protein synthesis and respiration.

It is clear, therefore, that the somewhat unexpected effects of potassium and calcium salt concentration on respiration and synthesis of protein become intelligible when it is recognized that the active ions are the cations and these exert their effect through the nitrogen metabolism and especially the metabolism of amino acids. The nitrate series confirm this view, since the combined effect of nitrate and potassium increases the metabolism of amino acids whereas the combined effect of calcium and nitrate tends to divert most of the nitrogen metabolism which does occur through channels

effects of potassium and calcium—is governed by the *ratio* between the concentration of the effective ions, rather than their total concentration in single salt solutions, and comparatively small concentrations of one ion (*e.g.*, Ca) may neutralize the effect of greater concentrations of the other (*e.g.*, K). The opposed effects of potassium and calcium ions in stoichiometrically equivalent concentrations must be of quite another kind. In the concept of antagonism there is the implication that single salt solutions are injurious and it is this injury which is suppressed by the antagonistic effect of ions of another kind. The evidence is that the potato discs in aerated, single-salt solutions are free from the suggestion of injury. In the greatest potassium concentrations the most protein was synthesized—a property hardly consistent with injury to the living system. Furthermore not a single sample *lost* fresh weight during the treatment. Though not separately recorded the observed gain of fresh weight in discs which were previously washed 24 hours in running tap water was never less than 3.7 per cent., even in the strongest solutions used; in the more dilute solutions, the gains were very much greater than those recorded.

The cations and not the anions are primarily effective. The anions play, however, an undoubted rôle in so far as they accentuate the effects of the associated cation and the relative order ($SO_4 < Br < Cl < NO_3$) in which they do this is clearly that in which they also promote absorption from salts of a common cation. The cations (K and Ca) exert their effect as they accentuate or depress reactions which involve free oxygen. The connection between respiration and protein synthesis of potato discs has already been discerned in the metabolism of potato discs and the response due to salts with different concentration only becomes intelligible when this connection is appreciated. As described, the metabolic effects due to oxygen, potassium, calcium, and nitrate all fall into one scheme in which the utilization of amino acids, after oxidative deamination in protein synthesis, plays a predominant rôle.

Clearly such effects of salt concentration may not be universal. In fact, the suggestion made that the salts act through the oxidase system of the potato might suggest that similar effects would be confined to the "direct" and not given by the "indirect" oxidase (peroxidase) plants. Not all living cells are capable of such active protein synthesis as are those of potato tuber. Hence it is not to be expected that so much of the aerobic respiration will always be linked with nitrogen metabolism.[12] Nevertheless, the results

[12] The rapid salt accumulation which can proceed in "low-salt" plants, apparently independently of growth, calls for separate treatment. It happens that the cases studied have been monocotyledons low in soluble nitrogen. For various reasons, protein synthesis in the cells under the conditions of experimentation is rather improbable. Some other aerobic process, however, plays the rôle in these plants which is similar to that played in the potato by the amino acids. The details are unknown but the approach is clear.

described, which connect three of the most fundamental properties of living cells (aerobic respiration, protein synthesis, and accumulation of potassium and calcium salts) in a manner which was not hitherto evident, have widespread implications.

Salt effects on potato discs and the Lundegårdh hypothesis

The results bear on the views of Lundegårdh (**8, 11, 12**) which have since been extended (**9**).[13] Criticisms of this hypothesis arise (**6**), from a consideration of evidence drawn from a diversity of plants, materials, and the work of many investigators. That the connection between salt uptake and respiration is a respiratory component, the very existence of which depends on anion absorption, has been denied[14] (**18**). Salt uptake responds to changes in the aerobic respiration rate (brought about by variables such as oxygen concentration, temperature, etc., other than anion absorption) so as to suggest that the one (respiration) regulates the other (salt absorption). Moreover, it was stated that, apart from the anions NO_3 and PO_4, the effects of salts on respiration were due chiefly to the cations—a possibility which Lundegårdh (**8, 12**) did not allow. Lundegårdh then, as now (**9**), postulated an entirely different mechanism to account for the absorption of anions and cations.

The earlier criticisms of Lundegårdh's theory gains added force from these new results. Lundegårdh evades the detailed criticisms of his earlier work but, at least to his satisfaction, "disposes of" the views of the senior author by the mere statement that potato discs constitute "unsuitable material" in which "anion respiration" is small relative to the "basic respiration" and the experimental conditions were varied too little in relation to the salts. The latter criticism relative to these experiments is clearly void, and the former is meaningless because as conceived by Lundegårdh "Anionenatmung" has no real existence in the respiration of potato discs—the demonstrable effects of neutral salts on respiration are due primarily to the cations.

In the case of potato discs rich in soluble nitrogen compounds, these amino acids occupy the key positions. In the work of Hoagland, however, it is shown that "low-salt" barley roots (which are probably unable to synthesize protein) are rich in sugar and organic acids; the influence of the latter predominates in this case. If the deamination of amino acids in potato discs affects respiration mainly because this yields a carbon substrate (keto acids), these two cases may not be as different as one might suppose. As a result of previous nutritional conditions, the substances in question may have already accumulated in the "low-salt" barley roots.

[13] Lundegårdh attributes the relation of respiration and salt absorption ($Rt = Rg + KA$) to a special component termed "Anionenatmung, KA" which is superimposed upon the basic respiration, "Grundatmung, Rg." "Anionenatmung" has a value proportional to the equivalents of anion absorbed and the constant of proportionality (K) has the values $NO_3 = 2$, $Cl = 3$.

[14] See also Hoagland and Broyer (**5**) and the later exchange of views (**7, 10, 11**).

Clearly constants characteristic of each anion could not account quantitatively for the effect of potassium and calcium salts on respiration; the effects due to the same anion supplied as a potassium or calcium salt are even different in *sign*. It is true that LUNDEGÅRDH has modified slightly his earlier view and now recognizes that "at more intense anion absorption" the cation affects *slightly* the value of the "constant" from which "Anionenatmung" is calculated. The mechanism postulated is a vaguely specified colloidal effect which is not directly determined by the quantity of cation absorbed.

If the total respiration of potato discs is to be partitioned at all, then the components which should be separated are those described earlier (**21**); namely, that part of the total respiration which in its production is linked with protein synthesis (and in special cases this may constitute as much as 43 per cent. of the total[15]) and the remainder which *arises independently of a net increase in protein and of nitrogen metabolism.* Whatever the final conclusion concerning the nature of the latter component—the data in this paper establish the reality of the former. The chief interest of such a partition of the total respiration is the evident fact *that the component of respiration which is linked to protein synthesis in potato discs seems to be more closely associated with salt accumulation than even the total respiration.* Since this component is entirely aerobic there is no discrepancy between this standpoint and the previous emphasis upon the importance of the aerobic processes of metabolism in salt accumulation. The new facts indicate that the aerobic metabolism processes in question are related to the nature and concentration of the cations in the external solution.

Further discussion is not necessary regarding LUNDEGÅRDH's views on the comparative rates of respiration of cells in distilled water and salt solution. Faced with the perhaps unexpected result (which does not follow from the theory of "Anionenatmung") that the calculated value of "Rg" is *less* than the respiration in distilled water (where anion absorption is clearly zero), LUNDEGÅRDH postulated that the concentration gradient is steeper in the absence of absorbable anions and more respiratory energy must be expended to retain the anions of the sap. He also stated that the presence of even a low external concentration of anion eliminates this necessity; in stronger solutions the "work of transport" rises and hence the increase in total respiration. In other words, the basic respiration is affected by the concentration gradient between solution and cell only when this latter is maximal (concentration difference = the concentration of the cell sap); at all other concentrations, and whenever absorption is finite, the magnitude of the respiration component "Rg" is independent of this factor. It is better to question the validity of the relationship Rt = Rg + KA than to

[15] Solution 4, KNO_3 series, for example.

adopt such an argument. As it concerns absorption, LUNDEGÅRDH's theory is not based upon the general concept that the work done in accumulation is a function of the concentration gradient against which the absorption occurs; and still less, upon any rigorous thermodynamic principles by means of which the work or energy value of the absorption can be evaluated.

The data in this paper need no such complicated assumptions regarding respiration in distilled water. For potato discs in distilled water respiration is at a level regulated solely by such variables as temperature, oxygen concentration, specific surface, etc.,—variables treated fully elsewhere—and in this condition the tissue is unaffected either by the stimulating effects of cations of one kind (*e.g.*, K) or the depressing effects of others (*e.g.*, Ca). In other words "respiration in distilled water" falls into its proper place in a range of salt treatments which passes from strong potassium salts on the one hand to strong calcium salts on the other.

What then measures the respiration of tissue not undergoing salt absorption according to LUNDEGÅRDH? LUNDEGÅRDH makes the assumption that there can be no anion absorption in bicarbonate solutions. Although this anticipates the results of another investigation similar to this one, it is clear that the bicarbonate ion exerts its own specific effects on metabolism. These—like the ones already described—are related to absorption. Other work by HOAGLAND, privately communicated to the authors, also shows that potassium cations are absorbed from bicarbonate solutions. This latter criticism would perhaps not be of such great importance were it not that LUNDEGÅRDH uses the respiration of roots at zero anion absorption to calibrate the respiratory efficiency of different batches of roots and, by the use of calculated factors, attains the semblance of close agreement between what are in reality a number of separate and obviously variable experiments. That the methods used by LUNDEGÅRDH still fail to standardize the variables in the material is evident from even a casual perusal of the absolute data; thus comparisons, except between the behavior of strictly comparable samples drawn from the same large batch of seedlings, could be justified only by a statistical analysis which is lacking. For all these reasons the theory of "Anionenatmung" is still to be regarded as unproved, relative to roots; with respect to potato discs, however, it is inconsistent with the established facts.

The recently published work of VAN EIJK (**22**) contributes the interesting result that the respiration of salt marsh plants (*Aster tripolium*) is much affected by salts (NaCl). He discusses the results from the standpoint of LUNDEGÅRDH but his calculated values for the constant anion respiration fluctuate over a wide range. His data do show that salts affect the respiration of halophytes; they do not establish the anion respiration as a special component of that respiration which is produced by salt absorption.

Relation between nitrogen metabolism and respiration

The case of barley plants and potato discs compared.—Gregory and Sen (3) recognized the possibility of a close connection between carbon dioxide output and nitrogen metabolism. In barley leaves the respiration was controlled by nutritional factors during development (potassium and nitrogen supply) and under these treatments high respiration was not associated with a high sugar content but rather with a high concentration of amino acids. The relatively low respiration of high-carbohydrate Lemna plants when grown under conditions of low nitrogen supply was similarly observed by White and Templeman (23). Discussing later experiments concerned with the effect of phosphorus nutrition on the composition and respiration of barley leaves, Richards (15) stressed mainly a supposed reciprocal relationship between carbon dioxide output and protein content. This relationship is regarded from a novel standpoint: not from the more familiar and old idea that protein is a measure of the total active substance capable of respiration, but from the converse standpoint that a certain fixed and continuous production of carbon dioxide is required to maintain a given amount of protein.

The results of the experiments on the effect of salts and oxygen on potato discs agree with the recent work on barley plants, in so far as respiration is not determined by sugar concentration. The behavior of potato discs suggests, however, that *neither* the concentration of amino acids nor of protein regulates the respiration rate and that the portion of the total carbon dioxide output which is linked with nitrogen metabolism is determined by the *conversion of amino acids to protein*. There is as yet no evidence for potato discs which indicates that the *concentration* of amino acids regulates protein synthesis (13); on the contrary, it appears to be regulated mainly by other factors (oxygen tension and the presence of inorganic cations) which determine the activity of systems in the cells that catalyze oxidation and also deaminate amino acids. Pearsall and Billimoria (14) rightly emphasize the diversity of factors which govern protein synthesis in different species and the vital properties such as age in the case of leaves.

Both the system investigated and the method of approach of Gregory and his associates differ in important respects from the case of aerated potato discs in salt solutions and, therefore, too close a parallel should not be drawn. Gregory and his co-workers modified the composition of their plants by nutritional means and, after steady states were established, the respiration rates were determined during periods so short that the composition of the leaves did not change appreciably. The rates observed were, therefore, regarded as regulated by the initial composition. Actual changes in the nitrogenous fractions associated with a given amount of carbon dioxide respired were apparently not investigated. In fact, it is implicit in

the scheme suggested that for the steady state net changes in the nitrogenous fractions would not be observed but that nevertheless the carbon dioxide output would be regulated by the continuous cycle of protein synthesis, breakdown, and resynthesis. The rate of the postulated cyclical process was conceived to be regulated apparently by the steady concentrations of the nitrogenous compounds of the cells caused by the nutritional treatments applied.

During development and storage the potato tuber does attain a steady level of soluble nitrogen compounds—a level which in GREGORY'S scheme might appear as the resultant of balanced tendencies to synthesis and breakdown. The conditions which obtain in the aerated cut discs, however, so encourage synthesis that any hypothetical tendency to simultaneous breakdown is masked and evidence of a continuous cycle of nitrogen metabolism is lacking. Under such conditions it is the new external conditions imposed which determine the response of the cut discs and their initial uniform composition, with reference either to salts or metabolites is *not* a determining factor. In fact, in so far as the metabolism is regulated by salts, it seems that *during entry these have access to more potent spheres of influence than the ions already stored in the cells* since their effects are disproportionately large relative to the amounts actually absorbed. This is in harmony with the idea—inescapable from these investigations—that salt uptake and protein synthesis of potato discs are processes which are linked together.

Protein breakdown is a conspicuous feature of mature leaves (**14**), especially of monocotyledons which are incapable of further growth; in the aerated potato discs, however, synthesis may be long maintained. In short, the nitrogen metabolism of the rapidly metabolizing cells of the potato discs does not appear as a cyclical process in which the rate is regulated by the steady concentrations of the components as envisaged by GREGORY and SEN (**3**), but rather as a linear one in which there is a progressive gain of protein at the expense of a reserve supply of amino acids and a steady state is not established. Other data show that this condition may be long maintained—in fact until most of the soluble nitrogen is converted to protein.[16] Potato cells are able to grow quite effectively in contrast with isolated or mature leaves of grasses. For this reason, considering the relation of respiration to nitrogen metabolism, greater emphasis must be placed upon the one-way process of synthesis than upon a hypothetical cycle of balanced synthesis and breakdown since the former can be demonstrated. It may be assumed with confidence that similar considerations will apply to other actively growing cells. The chief charm of the cyclical explanation is the ease with which, at some point, it can be adapted to demands made upon it;

[16] Thin potato discs in air will increase in protein content until this equals over 60 per cent. of the total nitrogen.

but this should not obscure the fact that it stands or falls by the evidence for the continuous cycle of protein synthesis and breakdown. It is precisely in those cases where cells are no longer able to grow[17] that the evidence for continuous breakdown must appear strongest. Also in the case here described nitrogen metabolism and respiration alike are regulated not by the concentrations of any nitrogenous or carbohydrate components of the tissue but by those external variables (oxygen and salt supply) which limit the extent to which the cells exercise their evident capacity for synthesis. This capacity is determined ultimately by the vital properties of the system concerned and not by the concentrations of any of the nitrogenous components. With these reservations, the views of GREGORY and SEN go far toward a general explanation of the data herein presented.

Accordingly, the interpretation of these results must rest upon a direct connection between protein synthesis and respiration. This connection becomes credible when it is appreciated that the stored amino acids may not be directly used in protein synthesis but first liberate ammonia which, with products derived from sugar, generates protein. Such views impinge upon old, and still open, controversies regarding the true nature of the relationship of both respiration and amino acids to protein synthesis and breakdown. Further reference should be made to the recent book by CHIBNALL (2) as extended reference to them is not possible here. The main conclusions, however, can rest upon the demonstrable facts that carbon dioxide output and the utilization of amino nitrogen in protein synthesis are inseparably linked in potato discs. Furthermore, both processes are regulated by variables (oxygen and the presence of salts) which determine the activity in the tissue of an enzyme system which can liberate ammonia from amino acids in vitro. That the corresponding α-keto-acids thus liberated contribute to the carbon substrates from which the increased carbon dioxide output is derived is an hypothesis which can be justified at present only by its probability.

The evident connection between protein synthesis and accumulation, which is the logical sequel to the parallelism between accumulation and growth previously noted, invites speculation on the mechanism of salt uptake. The obvious mode of approach is to postulate loose anion and cation combinations with the positive and negative ions of amphoteric amino acids. In this form the ions might be conducted across the protoplast only to be released into the vacuole when many of the acid and basic groups disappear

[17] CHIBNALL (2) in his recent book concludes that some factor from the root, probably hormonic, regulates the protein level of attached leaves. This is another way of saying that the response of attached leaves as part of the integrated system of shoot and root is different from those detached—a difference which seems determined by their capacity to grow whether this in turn is regulated by hormones or other properties of the organized system of which they form a part.

with synthesis. Until the nature of such postulated compounds of salts with amino acids is exactly specified, until the milieu in which synthesis occurs can be designated, and until the reason why the same mechanism does not equally conduct salts outward is clear, any attempt to build a theory of salt uptake on such premises is unwarranted.

Without the implication of the importance of synthesis BROOKS (1) has postulated anion and cation combinations with the proteins of the protoplasm, in which phase he believes the primary accumulation occurs. LUNDEGÅRDH (9) envisages the entry of anions (A) in combination with unspecified organic bases (R) and the essential stage of accumulation to occur at the inner surface when the compound (RA) activates the breakdown of glucose. While it may seem that there is a common element in these three views it must be clearly recognized however, that all such facile speculation is premature. Despite the evident effects of salt concentration on total uptake, protein synthesis, and respiration direct quantitative relations between them are elusive. In fact, it appears from a consideration of the results that such cannot be expected. The essential point is that *accumulation in potato discs occurs only if protein synthesis takes place* but mere passive absorption to equality of concentration can occur irrespective of protein synthesis. It remains for future work to specify more closely the contact between salt accumulation and the aerobic synthesis of protein from amino acids and to distinguish between effects which are due to the energy value of the extra respiration thus stimulated, effects which could be produced by any other substrate of aerobic respiration; and those which are peculiarly dependent on protein synthesis because of some essential reactions which this entails.

Summary

1. The effect of a range of salt concentrations on respiration and metabolism of potato discs is described. The experiments were carried out under controlled conditions conducive to salt accumulation. The salts used were bromides, chlorides, and nitrates of potassium; and calcium and potassium sulphates. The carbon dioxide output of the discs and the effect of the treatment on carbohydrate, soluble nitrogen fractions, amino and amide nitrogen, ammonia, and protein nitrogen were determined.

2. The effects of salt concentration on starch hydrolysis are slight. The tendency is for increased concentration of potassium salts to decrease and calcium salts to increase starch hydrolysis. This is the converse of the more important effect of salts on all the processes which involve oxidation.

3. Increased external concentration of potassium salts increases respiration and all other reactions which are favored by oxygen, whereas corresponding concentrations of the calcium salts with a common anion depress these processes.

4. The salt concentrations which induce high respiration do not produce high sugar content. High rates of respiration and lower sugar content obtain in the tissue exposed to strong potassium salts and the converse is true of the discs treated with calcium salts. Sugar concentration does not determine respiration.

5. The effective ions of the salts are the cations. The specific effects of the cations are accentuated by the anions and these, like the contrast between the effect of potassium and calcium salts at the same equivalent concentration, are influenced by the anions in the order $NO_3 > Cl > Br > SO_4$ which is also the order in which they influence absorption of a common cation.

6. The effects of salts on respiration are closely connected with their effect on protein synthesis from stored amino acids. Potassium salts stimulate, calcium salts depress both processes.

7. In potassium nitrate solutions the bulk of the nitrate absorbed passes via amino acid to protein. In calcium nitrate solutions less nitrate is absorbed, much less of it appears as protein and the nitrogen metabolism is diverted from amino acids to amide and ammonia.

8. Only the protein synthesis which proceeds from amino acids is linked to respiration. The contact between protein synthesis and respiration seems to be the oxidative deamination of amino acid. Amino acids do not yield protein directly but are first deaminated; they then release ammonia and a carbon residue (probably keto-acid) which contributes to the substrates of aerobic respiration. The activity of the oxidase system of potato which produces oxidizing agents which deaminate amino acids is stimulated by the salts that also increase protein synthesis.

9. Amides, easily hydrolyzed at pH 6.5 in hot solutions, the stability of which is similar to that of glutamine, accumulate in the actively respiring tissue and appear to be the products derived from sugar and ammonia from which protein is formed.

10. Protein synthesis and the aerobic respiration with which it is associated are the phases of the metabolism of potato discs most closely concerned with salt accumulation. Accumulation occurs even in strong (0.075 equivalents per liter) solutions of potassium salts but in calcium salts of this strength, in which a net gain of protein is not produced, the absorption of anions does not exceed equality with the external solution.

11. The salt effects are exerted in centers accessible to salts being absorbed; these centers, however, are inaccessible to salt merely present in the external solution or accumulated in the sap.

12. The bearing of the results upon the present status of the problem of salt accumulation is discussed. The necessity for further work to elaborate the connection between salt absorption, protein synthesis, deamination of

amino acids, and the aerobic respiration with which it is associated, is emphasized.

12. Comparison is made between the relation of respiration to nitrogen metabolism as revealed by work on mature barley leaves and potato discs.

13. The data are discussed relative to LUNDEGÅRDH's theory, to which exception is taken.

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FOLIAR DIAGNOSIS STUDY OF CLIMATIC INFUENCES ON THE NUTRITION OF SPRING AND FALL GROWN GREENHOUSE TOMATOES[1]

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(WITH THIRTEEN FIGURES)

Introduction

Up to the present, our studies on foliar diagnosis (**4, 5, 7, 8, 9, 10**) have been confined to the effect of fertilizers acting during a particular growing season. But the effect of a specific fertilizer element on growth and development is the resultant action of (1) the fertilizer itself and also (2) that of the medium; that is, of the unfertilized soil together with the meteorological factors.

The physical characteristics constituting the meteorological forces can influence growth and development, either *directly* by action on the aerial portions of the plant or *indirectly* by their effect on the chemical, biological, and physical condition of the soil. Accordingly, with respect to the chemical processes taking place in the leaf, there exists what may be conveniently designated a "chemism," due to the impulse given by the fertilizer itself, and also a "chemism" due to the impulses given by the meteorological factors (**1**).

Inasmuch as the same fertilizer element may result, in meteorologically different years, in giving widely different developments and yields, it is necessary to postulate that the influence of a given fertilizer element on the mode of nutrition of a given species is different in meteorologically different years or seasons.

Experimental evidence in support of such an hypothesis has been established (**1**), the theoretical considerations for which are deduced in terms of the concepts of the method of foliar diagnosis and applied to the conditions of the present investigation in the experimental part of this paper.

The purpose of this investigation is to obtain information on the relative magnitude of the differences in the foliar diagnosis of a particular plant species and variety subjected to differential fertilizer treatment under such extreme differences in the climatic conditions as exist in the spring and fall of a particular year. Under the particular conditions of this experiment the principal differences in climatic conditions are those due to differences in intensity and daily duration of light in the spring and in the fall, respectively.

[1] Authorized for publication as Paper no. 953 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

Materials and methods

Descriptions of the experimental lay-out and methods of culture have been given previously (**2, 10**). For purposes of orienting the reader the following facts pertinent to the experiment are given.

The plots, twenty in number, were 5¾ feet wide by 8 feet long and contained 12 plants each. The soil was a composted clay loam about 14 inches deep lying over a clay loam subsoil and was supplied with lines of 4-inch drain tiles, 12 inches below the surface and 27 inches apart. The fertilizer treatments, shown in table I, consisted of single elements, combinations of two elements, and also of all three elements, one series with and another without manure. Nitrogen was supplied in the form of sodium nitrate equivalent to 42.1 gm. of N to each plot;[2] phosphoric acid in the form of

TABLE I

PLOT NUMBERS WITH TREATMENTS AND YIELDS OF FRUIT

Plot	Treatment	Symbol	Yield	
			Spring	Fall
			lb.	*lb.*
10R	Nothing	Check	88.1	54.7
10L	Manure	M	113.2	63.2
12L	NaNO	N	64.4	54.6
12R	$NaNO_3$ + manure	N + M	121.9	60.1
14L	Superphosphate	P	43.3	64.9
14R	Superphosphate + manure	P + M	113.5	53.6
16L	$NaNO_3$ Superphosphate	NP	30.9	70.2
16R	" + manure	NP + M	117.9	71.3
18L	$NaNO_3$ KCl	NK	95.6	67.3
18R	" + manure	NK + M	120.5	68.1
20L	Superphosphate KCl	PK	98.8	70.5
20R	" + manure	PK + M	129.3	85.2
2R	$NaNO_3$ Superphosphate KCl	NPK	113.8	60.7
2L	" + manure	NPK + M	121.8	65.1
4R	$NaNO_3$ Superphosphate KCl	(2N)PK	107.3	62.3
4L	" + manure	(2N)PK + M	115.5	67.1
8R	$NaNO_3$ Superphosphate KCl	(RN)PK	104.5	62.6
8L	" + manure	(RN)PK + M	120.5	60.9

[2] Plots 4R and 4L received twice this amount of N and plots 8R and 8L, biweekly additions of the unit quantity of N.

superphosphate equivalent to 125.2 gm. of P_2O_5 per plot, and potash as the muriate, equivalent to 43.1 gm. of K_2O per plot, were applied just before planting. These applications are equivalent to 500 lb. of $NaNO_3$, 1250 lb. of superphosphate, and 350 lb. of muriate of potash to the acre.

Manure applications consisted of 110 lb. of rotted horse manure per plot (about 50 tons to the acre) spaded into the soil just before steam pasteurization, preparatory to the planting of the fall crop. The amounts of manure applied to each plot contained 422.1 gm. of N, 274.9 gm. of P_2O_5, and 345.8 gm. of K_2O. The plants, which were grown from Pennsylvania certified seed of the Marglobe variety, were transplanted from 4-inch pots into the beds on March 9 for the spring crop and on August 15 for the fall crop.

Pollination was by means of a watch glass applied to the stigmas of well-opened flowers. Up until the plants were about 4 feet tall they were sprayed with cuprous oxide suspension in water, at the rate of one pound of cuprous oxide to 50 gallons of water, with a neutral wetting agent. Water was supplied by allowing it to run from a hose for the same length of time on each plot. Fruits were harvested when well-colored, and were weighed immediately. The treatment given to each plot is shown in table I.

Temperatures were maintained according to the usual practice in commercial greenhouses; from 60° to 65° F. at night, 65° to 70° on cloudy days, and approximately 75° on clear days unless the temperature rose above these ranges without artificial heat and with the ventilators open. The average daily mean temperature, computed from continuous thermograph records, was 74.8° for the spring crop and 73.5° for the fall crop.

Results

The yields obtained from the spring and fall crop, respectively, are shown in table I, and the percentages of nitrogen, phosphoric acid, and potash in the dried foliage at the three dates of sampling, together with the milligram equivalent values and the composition of the NPK-units, are shown in table II and presented graphically in figures 1 to 12.

Discussion of results

Comparison of the yields from each treatment under spring and fall climatic conditions

In the manured series the yield from a particular plot is always much greater in the spring than in the fall; in the majority of treatments it is about twice as great in the spring.

In the unmanured series the yield from a particular plot is also higher in the spring than in the fall except in two treatments, [P] and [NP], that resulted in a severe attack of fusarium wilt of tomato in the spring. In the unmanured series, however (with the exception of the plots mentioned), the

TABLE II

THE PERCENTAGE OF THE N, P_2O_5, AND K_2O IN THE DRIED FOLIAGE, MILLIGRAM EQUIVALENTS, AND THE COMPOSITION OF THE NPK-UNITS OF THE FIFTH LEAF IN THE SPRING AND FALL OF PLANTS RECEIVING TREATMENTS INDICATED WITHOUT MANURE

Date of sampling	Mineral content of dried foliage				Milligram equivalents				Composition of NPK-units		
	N	P_2O_5	K_2O	$N + P_2O_5 + K_2O$	N	P_2O_5	K_2O	S	X	Y	Z
	(M_X)	(M_Y)	(M_Z)	($M_X + M_Y + M_Z$)	(E_X)	(E_Y)	(E_Z)	$E_X + E_Y + E_Z$	$\left(100\frac{E_X}{S}\right)$	$\left(100\frac{E_Y}{S}\right)$	$\left(100\frac{E_Z}{S}\right)$
	Nothing (Plot 10 R)										
	%	%	%	%	m.e.	m.e.	m.e.	m.e.			
April 5	3.960	1.871	1.495	7.326	282.744	79.143	31.843	393.730	71.811	20.101	8.087
April 29	2.900	2.500	0.689	6.089	207.060	105.750	14.075	327.485	63.227	32.291	4.481
May 27	2.140	3.066	0.646	5.852	152.796	129.691	13.759	296.246	51.577	43.778	4.644
Sept. 3	4.840	1.520	1.292	7.652	345.576	64.296	27.519	437.391	79.008	14.699	6.291
Sept. 23	3.660	1.846	0.904	6.410	261.324	78.086	19.255	358.665	72.860	21.771	5.368
Oct. 21	2.900	2.240	0.629	5.769	207.060	94.752	13.398	315.210	65.686	30.059	4.254
	N (Plot 12 L)										
April 5	4.420	1.180	1.455	7.055	315.588	49.914	30.991	396.493	79.592	12.589	7.817
April 29	3.140	1.600	0.835	5.575	224.196	67.680	17.785	309.661	72.404	21.856	5.740
May 27	2.520	1.840	0.671	5.031	179.928	77.832	14.292	272.052	66.137	28.608	5.253
Sept. 3	4.660	1.400	1.356	7.416	332.724	59.220	28.882	420.826	79.064	14.072	6.863
Sept. 23	3.820	1.866	1.208	7.894	272.748	78.932	25.730	377.410	72.268	20.914	6.817
Oct. 21	3.260	2.173	0.872	6.305	232.764	91.918	18.573	343.255	67.810	26.778	5.410
	P (Plot 14 L)										
April 5	3.900	1.886	0.852	6.638	278.460	79.778	18.147	376.385	73.983	21.196	4.821
April 29	2.380	2.386	0.667	5.433	169.932	100.928	14.207	285.067	59.611	35.405	4.983
May 27	1.840	2.826	0.646	5.312	131.376	119.540	13.760	264.676	49.614	45.183	5.202
Sept. 3	4.900	1.606	1.705	8.211	349.860	67.934	36.316	454.110	77.043	14.959	7.997
Sept. 23	4.020	1.720	1.389	7.129	287.028	72.756	29.585	389.369	73.719	18.686	7.595
Oct. 21	3.140	1.906	1.001	6.047	224.196	80.624	21.321	326.141	68.742	24.720	6.537

TABLE II—(*Continued*)

Date of Sampling	Mineral content of dried foliage				Milligram equivalents				Composition of NPK-units		
	N (M_X)	P_2O_5 (M_Y)	K_2O (M_Z)	$N+P_2O_5+K_2O$ ($M_X+M_Y+M_Z$)	N (E_X)	P_2O_5 (E_Y)	K_2O (E_Z)	S ($E_X+E_Y+E_Z$)	X $\left(100\frac{E_X}{S}\right)$	Y $\left(100\frac{E_Y}{S}\right)$	Z $\left(100\frac{E_Z}{S}\right)$
	NP (Plot 16 L)										
	%	%	%	%	m.e.	m.e.	m.e.	m.e.			
April 5	4.440	1.473	1.137	7.050	317.016	62.308	24.218	403.542	78.559	15.440	6.001
April 29	2.740	1.920	0.665	5.325	202.776	81.216	14.164	298.156	68.010	27.239	4.750
May 27	2.000	2.393	0.594	4.987	142.800	101.224	12.652	256.676	55.634	39.436	4.929
Sept. 3	4.780	1.340	2.031	8.151	341.292	56.682	43.260	441.234	77.349	12.846	9.805
Sept. 23	4.000	1.520	1.744	7.264	285.600	64.296	22.447	372.343	76.703	17.268	6.028
Oct. 21	3.500	1.360	1.388	6.248	249.900	57.528	29.564	336.992	74.155	17.071	8.773
	NK (Plot 18 L)										
April 5	4.520	1.113	2.067	7.700	322.728	47.080	44.027	413.835	77.984	11.376	10.638
April 29	3.080	1.113	1.001	5.194	219.912	47.080	21.321	288.313	76.275	16.329	7.395
May 27	2.420	1.313	0.775	4.508	172.788	55.540	16.507	244.835	70.573	22.684	6.741
Sept. 3	5.060	1.260	1.957	8.277	361.284	53.298	41.684	456.266	79.182	11.681	9.135
Sept. 23	4.280	1.556	1.337	7.173	305.592	65.819	28.418	399.887	76.419	16.459	7.121
Oct. 21	3.540	1.666	0.972	6.178	252.756	70.471	20.703	343.930	73.490	20.489	6.019
	PK (Plot 20 L)										
April 5	3.680	1.393	2.099	7.172	262.752	58.924	44.708	366.384	71.715	16.081	12.202
April 29	2.200	1.773	1.292	5.265	157.080	75.000	27.518	259.598	60.509	28.891	10.600
May 27	1.700	1.960	1.033	4.693	121.380	82.908	22.003	226.291	53.638	36.637	9.723
Sept. 3	5.020	1.513	2.733	9.266	358.428	64.000	58.212	480.640	74.573	13.315	12.111
Sept. 23	3.740	1.820	1.841	7.401	267.036	76.986	39.213	383.235	69.679	20.088	10.232
Oct. 21	3.300	1.926	1.453	6.679	235.820	80.469	30.949	347.238	67.913	23.174	8.912

TABLE II—(*Continued*)

Date of sampling	Mineral content of dried foliage: N (M_X)	P$_2$O$_5$ (M_Y)	K$_2$O (M_Z)	N + P$_2$O$_5$ + K$_2$O ($M_X + M_Y + M_Z$)	Milligram equivalents: N (E_X)	P$_2$O$_5$ (E_Y)	K$_2$O (E_Z)	S ($E_X + E_Y + E_Z$)	Composition of NPK units: X ($100\frac{E_X}{S}$)	Y ($100\frac{E_Y}{S}$)	Z ($100\frac{E_Z}{S}$)
					NPK (Plot 2 R)						
	%	%	%	%	*m.e.*	*m.e.*	*m.e.*	*m.e.*			
April 5	4.360	1.230	1.783	7.373	311.304	52.029	37.978	401.311	77.571	12.964	9.463
April 29	3.180	1.560	1.076	5.816	227.052	65.988	22.919	315.959	71.861	20.885	7.253
May 27	2.400	1.893	0.826	5.119	171.360	80.074	17.594	269.028	63.695	29.764	6.539
Sept. 3	4.920	1.460	1.841	8.221	351.288	61.758	39.213	452.259	77.674	13.655	8.670
Sept. 23	3.820	1.760	1.712	7.292	272.748	74.448	36.465	383.748	71.090	19.404	9.504
Oct. 21	3.260	1.786	1.066	6.112	232.764	75.548	22.706	331.018	70.317	22.822	6.859
					(2N)PK (Plot 4 R)						
April 5	4.600	1.213	1.873	7.686	328.440	51.309	39.895	419.644	78.266	12.226	9.507
April 29	3.320	1.600	0.981	5.901	237.048	67.680	20.895	325.623	72.797	20.785	6.417
May 27	2.720	2.153	0.925	5.798	194.208	91.072	19.702	304.982	63.678	29.861	6.460
Sept. 3	5.220	1.340	1.550	8.110	372.708	56.685	33.015	462.405	80.602	12.258	7.139
Sept. 23	3.960	1.766	1.292	7.018	282.744	74.702	27.519	384.965	73.446	19.404	7.148
Oct. 21	3.400	1.746	0.839	5.985	242.760	73.856	17.871	334.487	72.576	22.081	5.342
					(RN)PK (Plot 8 R)						
April 5	4.820	1.226	2.164	8.210	344.148	51.859	46.093	442.100	77.843	11.730	10.425
April 29	3.880	1.320	1.309	6.509	277.032	55.836	27.881	360.749	76.793	15.478	7.728
May 27	3.080	1.640	0.861	5.581	219.912	69.372	18.339	307.623	71.487	22.551	5.961
Sept. 3	5.180	1.393	1.886	8.459	369.852	58.924	40.172	468.948	78.868	12.565	8.566
Sept. 23	4.080	1.713	1.337	7.130	291.312	72.460	28.478	392.250	74.266	18.473	7.260
Oct. 21	3.340	1.746	0.904	5.990	238.476	73.856	19.255	331.587	71.919	22.273	5.806

TABLE II—(*Continued*)

The percentage of N, P_2O_5, K_2O in the dried foliage, milligram equivalents, and the composition of the NPK-units of the fifth leaf in the spring and in the fall of plants receiving the treatments shown with manure

Date of sampling	Mineral content of dried foliage: N (M_X)	P_2O_5 (M_Y)	K_2O (M_Z)	$N+P_2O_5+K_2O$ ($M_X+M_Y+M_Z$)	Milligram equivalents: N (E_X)	P_2O_5 (E_Y)	K_2O (E_Z)	S ($E_X+E_Y+E_Z$)	Composition of NPK-units: X $\left(100\frac{E_X}{S}\right)$	Y $\left(100\frac{E_Y}{S}\right)$	Z $\left(100\frac{E_Z}{S}\right)$
					Manure (Plot 10 L)						
	%	%	%	%	*m.e.*	*m.e.*	*m.e.*	*m.e.*			
April 5	3.960	1.346	3.630	8.936	282.744	56.936	77.319	416.999	67.804	13.653	18.542
April 29	2.800	1.746	1.906	6.532	205.632	73.856	40.597	320.085	64.243	23.074	12.683
May 27	2.140	1.833	2.067	6.040	152.796	77.535	44.027	274.358	55.692	28.260	16.047
Sept. 3	4.860	1.480	3.068	9.308	347.004	62.604	65.348	347.004	73.060	13.181	13.758
Sept. 23	4.160	1.720	2.403	8.283	297.024	72.756	51.184	297.024	70.558	17.283	12.159
Oct. 21	3.560	1.893	2.261	7.714	254.184	80.074	48.159	254.184	66.467	20.938	12.593
					N + Manure (Plot 12 R)						
April 5	4.660	1.080	3.049	8.789	332.724	45.684	64.944	443.352	75.047	10.304	14.648
April 29	3.700	1.500	2.454	7.654	264.180	63.450	52.270	264.180	69.539	16.701	13.758
May 27	2.900	1.646	1.925	6.471	207.060	69.626	41.002	207.060	65.177	21.916	12.906
Sept. 3	5.040	1.340	3.078	9.458	359.856	56.682	65.561	482.099	74.643	11.757	13.599
Sept. 23	4.380	1.560	2.713	8.653	312.732	65.988	57.787	436.507	71.644	15.117	13.238
Oct. 21	3.580	1.792	2.196	7.568	255.612	75.802	46.775	378.189	67.588	20.043	12.367
					P + Manure (Plot 14 R)						
April 5	3.680	1.013	3.490	8.183	262.752	42.850	19.566	379.939	69.156	11.278	19.566
April 29	2.600	1.673	1.698	5.971	185.640	70.768	12.361	292.575	63.450	24.187	12.361
May 27	2.180	1.746	1.647	5.573	155.652	73.856	35.081	264.589	58.827	27.913	13.258
Sept. 3	5.440	1.393	3.488	10.321	388.416	58.924	74.294	521.635	74.461	11.296	14.242
Sept. 23	4.640	1.620	3.004	9.264	331.296	68.526	63.985	463.807	71.429	14.774	13.795
Oct. 21	3.600	1.900	2.506	8.006	257.040	80.370	53.378	257.040	65.774	20.566	13.659

TABLE II—(*Continued*)

Date of sampling	Mineral content of dried foliage: N (M_X)	P₂O₅ (M_Y)	K₂O (M_Z)	N + P₂O₅ + K₂O $(M_X + M_Y + M_Z)$	Milligram equivalents: N (E_X)	P₂O₅ (E_Y)	K₂O (E_Z)	S $E_X + E_Y + E_Z$	Composition of NPK-units: X $\left(100\frac{E_X}{S}\right)$	Y $\left(100\frac{E_Y}{S}\right)$	Z $\left(100\frac{E_Z}{S}\right)$
	NP + Manure (Plot 16 R)										
	%	%	%	%	*m.e.*	*m.e.*	*m.e.*	*m.e.*			
April 5	4.520	1.200	3.727	9.447	322.728	50.760	79.385	452.873	71.261	11.208	17.529
April 29	3.240	1.646	1.925	6.811	231.336	69.626	41.002	341.964	67.651	20.360	11.989
May 27	2.480	1.600	1.744	5.824	177.072	67.680	37.147	281.879	62.813	24.008	13.177
Sept. 3	5.200	1.313	3.359	9.872	371.280	55.540	71.546	498.366	74.499	11.144	14.356
Sept. 23	4.400	1.760	2.745	8.905	314.160	74.448	58.468	447.076	70.269	16.652	13.078
Oct. 21	3.500	1.886	2.681	8.067	249.900	79.778	57.105	386.783	64.609	20.626	14.764
	NK + Manure (Plot 18 R)										
April 5	4.660	1.313	2.196	8.169	332.724	55.539	46.774	435.037	76.481	12.766	10.751
April 29	3.640	1.453	2.648	7.741	259.896	61.462	56.402	377.760	68.799	16.270	14.930
May 27	3.080	2.173	2.648	7.901	219.912	91.918	56.402	368.232	59.721	24.962	15.317
Sept. 3	4.860	1.433	2.887	9.180	347.004	60.616	61.493	469.113	73.970	12 921	13.108
Sept. 23	4.280	1.720	3.068	9.068	305.592	72.756	65.348	443.696	68.874	16.397	14.728
Oct. 21	3.640	1.813	2.390	7.843	259.896	76.690	50.907	387.493	67.071	19.791	13.137
	PK + Manure (Plot 20 R)										
April 5	4.260	1.300	4.166	9.726	304.164	54.990	88.736	447.890	67.910	12.277	19.812
April 29	3.020	1.686	2.680	7.386	215.628	71.319	57.084	344.031	62.676	20.730	16.592
May 27	2.300	1.920	2.196	6.416	164.220	81.216	46.774	292.210	56.199	27.793	16.006
Sept. 3	4.900	1.366	3.844	10.110	349.860	57.782	81.877	489.519	71.470	11.824	16.705
Sept. 23	4.140	1.760	3.294	9.194	295.596	74.448	70.162	440.206	67.149	16.912	15.938
Oct. 21	3.260	1.913	2.584	7.757	232.764	80.820	55.039	368.623	63.144	21.924	14.931

TABLE II—(*Concluded*)

Date of Sampling	Mineral Content of Dried Foliage				Milligram Equivalents				Composition of NPK-units		
	N	P_2O_5	K_2O	$N + P_2O_5 + K_2O$	N	P_2O_5	K_2O	S	X	Y	Z
	(M_X)	(M_Y)	(M_Z)	$(M_X + M_Y + M_Z)$	(E_X)	(E_Y)	(E_Z)	$E_X + E_Y + E_Z$	$\left(100\frac{E_X}{S}\right)$	$\left(100\frac{E_Y}{S}\right)$	$\left(\frac{E_Z 100}{S}\right)$
					NPK + Manure (Plot 2 L)						
	%	%	%	%	*m.e.*	*m.e.*	*m.e.*	*m.e.*			
April 5	4.360	1.184	3.727	9.271	311.304	50.083	79 385	440.772	70.626	11.362	18.014
April 29	3.200	0.946	2.874	7.020	228.480	40.016	61.216	329.712	69.296	12.136	18.566
May 27	2.760	1.366	2.209	6.335	197.064	57.782	47.051	301.897	65.275	19.139	15.585
Sept. 3	5.260	1.286	3.333	9.879	375.564	54.398	70.993	500.955	74.969	10.585	14.171
Sept. 23	4.280	1.393	3.242	8.915	305.592	58.924	69.054	433.570	70.482	13.590	15.926
Oct. 21	3.480	1.732	2.648	7.860	248.472	73.264	56.264	378.138	65.709	19.375	14.915
					(2N)PK + Manure (Plot 4 L)						
April 5	4.540	1.240	2.842	8.622	324.156	52.452	60.534	437.142	74.153	11.998	13.847
April 29	3.460	1.480	2.704	7.644	247.044	62.604	57.595	367.243	67.269	17.046	15.684
May 27	2.800	1.386	1.563	5.749	191.920	58.628	33 292	291.840	68 503	20.089	11.407
Sept. 3	5.060	1.300	2.971	9.331	361.284	54.990	63 282	479.556	75.337	11.466	13.195
Sept. 23	4.220	1.480	2.680	8.380	301.308	62.604	57.084	420 996	71.570	14.870	13.559
Oct. 21	3.340	1.773	2.196	7.309	238 476	74.998	46 775	360.476	66.197	20.818	12.984
					(RN)PK + Manure (Plot 8 L)						
April 5	4.620	1.233	3.081	8.934	329.868	51.156	65.625	446.649	73.853	11.453	14.692
April 29	3.780	1.280	2.667	7.727	269.892	54.144	56.807	380.843	70.866	14.216	14.916
May 27	3.220	1.260	2.357	6.837	229.908	53.298	50.204	333.410	68.956	15.985	15.057
Sept. 3	5.260	1.340	3.320	9.920	375.564	56.682	70.716	502.962	74.670	11.271	14.059
Sept. 23	4.580	1.633	2.713	8.926	327.012	69.076	57.787	453.875	72.048	15.219	12.731
Oct. 21	3.640	1.740	1.550	6.930	259.896	73.602	33.015	366.513	70.910	20.081	9.007

differences between the yields in the two seasons from a particular treatment are not so great as the differences existing in the manured series.

Furthermore, the range of differences in yields from different treatments is much greater in the spring than in the fall.

THE COURSE OF NUTRITION AS INDICATED BY THE VALUES OF THE PERCENTAGES OF NITROGEN, PHOSPHORIC ACID, AND POTASH IN THE DRIED FOLIAGE AT THE THREE DATES OF SAMPLING

These values are recorded in the second, third, and fourth columns, respectively, of table II and are shown graphically in figure 1.

COMPARISON OF THE GRAPHS OF EACH TREATMENT UNDER SPRING AND FALL CONDITIONS RESPECTIVELY.—In both the manured and the unmanured series the nitrogen graph of a particular plot in the fall is always much above that of the same plot in the spring; this indicates that demand is greater relative to supply in the spring than in the fall.

At the first date of sampling the graph for the potash in the fall is frequently lower than that of the same plot in the spring; this is true of the [nothing], [N], [NK], [(2N)PK], and [(RN)PK] treatments in the unmanured series and of [m], [NP + m], [PK + m], and [NPK + m] in the manured series. At the second sampling, with increasing age of the leaves, however, the graph for potash of a particular plot in the fall is above that of the same plot in the spring with four exceptions: nothing and [(2N)PK] in the unmanured series, and [NK + m] and [(RN)PK + m] in the manured series; this condition holds for the third sampling also. These relations probably indicate that utilization of potash, or demand in relation to supply, is less in the fall than in the spring.

In the case of the phosphoric acid graphs no consistent relationship is apparent between the positions of the graphs of a particular plot in the spring and fall.

The nitrogen graphs in all cases and the potash graphs in all except [manure] in the third period in spring and [NK + m] in the fall descend with increasing maturity of the leaf. Furthermore, with the exception of the potash graphs of those plants that were non-resistant to fusarium wilt, [P] and [NP], they are steeper in the spring than in the fall; this indicates greater demand relative to supply for nitrogen and potash during the spring season.

On the other hand, the graphs of phosphoric acid ascend, in most cases progressively with increasing maturity of the leaf, in both spring and fall in every treatment, thus indicating that the supply of this element relative to demand of the growing plant is greater irrespective of the season. Since

this phenomenon also occurs to a striking degree in the check plot (plot no. 10R) which has received no fertilizer and no manure, and, moreover, is at a

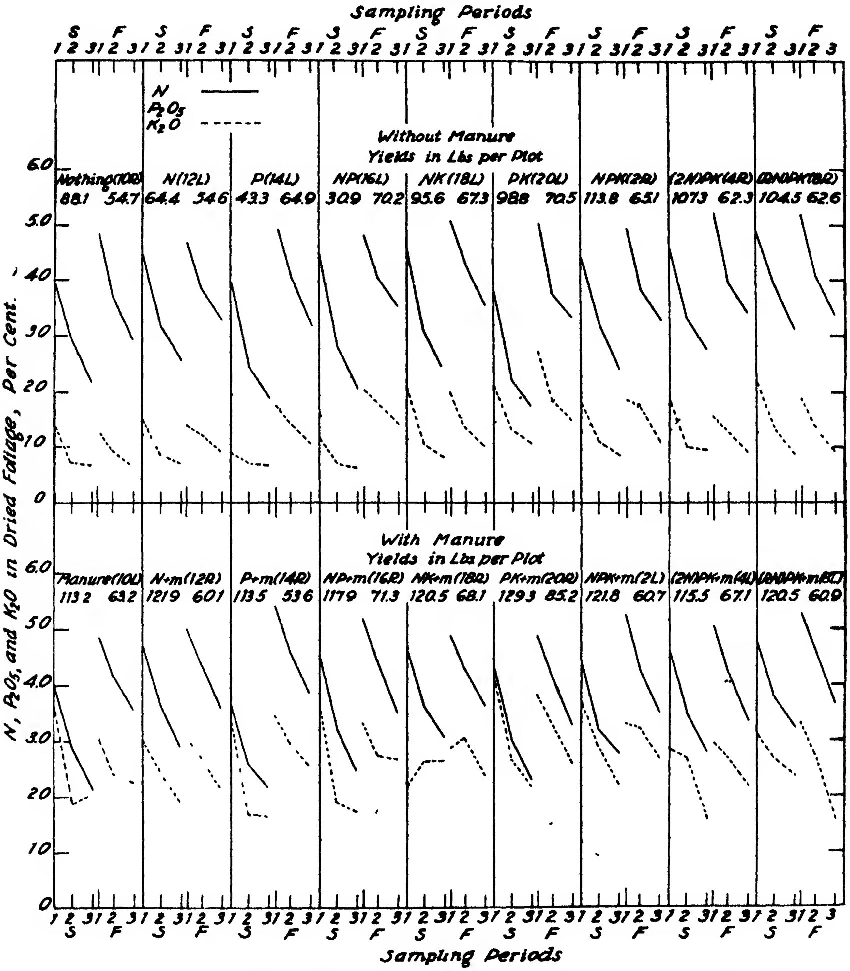

FIG. 1. Changes during the growth cycle in the content of N, P_2O_5, and K_2O in the fifth leaf in the spring and also in the fall of plants of the manured and unmanured series receiving the treatments indicated. Percentage values in terms of the dried foliage are the ordinates and dates of sampling are the abscissae.

relatively high level, one must conclude that this composted soil is able to supply phosphoric acid at a relatively rapid rate, especially during the spring season.

The course of nutrition as indicated by the values of the intensities of nutrition and the composition of the NPK-units at the three dates of sampling

Comparison of the graphs from each of the treatments under spring and fall conditions. *The general characteristics.*—With respect to

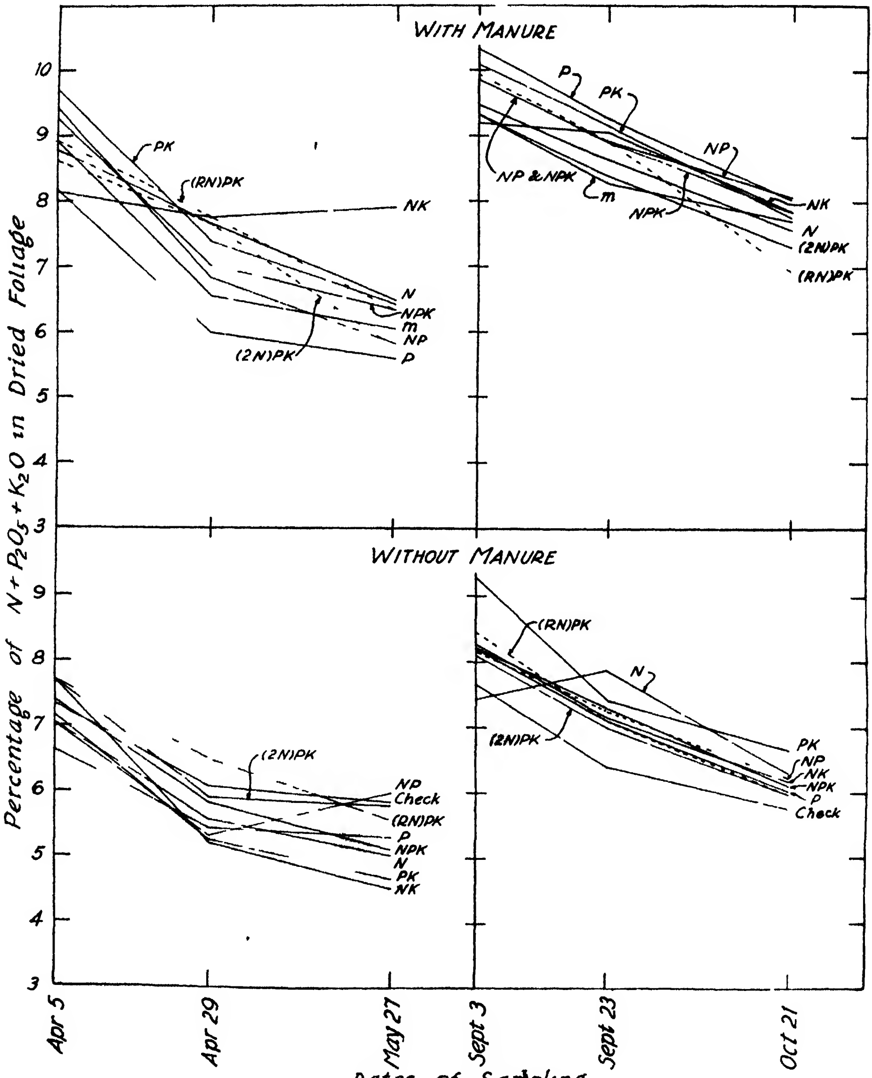

FIG. 2. Intensities of nutrition of the fifth leaf in the spring and also in the fall of plants of the manured and unmanured series receiving the treatments shown. Percentage values are the ordinates and dates of sampling are the abscissae.

the quantity factor of nutrition (**4**), in both the manured and unmanured series the intensity resulting from a particular treatment is higher in the fall than in the spring (fig. 2).

Figures 3 to 11 show that the graph of a particular plot is displaced higher towards the summit (N = 100 per cent.) of the triangle in the fall than in the spring and always during the latter part of the cycle; with few exceptions this holds throughout the cycle. The graph of a particular plot is, morever, with two exceptions shorter in the fall.

Foliar diagnosis studies of field crops have indicated that absorption of phosphoric acid increased with increase in temperature. The higher temperatures prevailing in the greenhouse in the spring afford an explanation of the relatively smaller displacement of the locus of the co-ordinate of a particular plot from the right base apex ($P_2O_5 = 100$) in the spring than in the fall. In the plots which received supplies of nitrogen larger than the unit amounts this phenomenon is masked by greater utilization of phosphoric acid brought about by the increase in the nitrogen nutrition.

The displacement of the graph of a particular plot with respect to the left base apex ($K_2O = 100$ per cent.) is farther away from this apex during most of the cycle in the fall relative to the graph of the same plot in the spring. The notable exceptions (as might be expected) are the three treatments which, in the spring, resulted in diseased conditions—*viz.*, [N] plot no. 12L, [P] plot no. 14L, and [NP] plot no. 16L.

Mechanism of the action of a particular element in meteorologically different years or seasons.—In the introduction to this paper it was stated that the fact that a fertilizer element resulted in different yields in meteorologically different seasons suggested a search for the manner in which a particular element acted in different seasons. Reasoning deductively from the principles established experimentally by means of the method of foliar diagnosis (**4**), we can explore the possible mechanism.

A particular fertilizer element, if effective, intervenes in the nutrition of a plant to effect an increase *of this element* either: (1) in the magnitude ($N + P_2O_5 + K_2O$), *i.e.*, the intensity of nutrition; or (2) in the composition of the *NPK-unit, i.e.,* in the equilibrium between $N–P_2O_5 – K_2O$; or (3) in both (1) and (2) simultaneously. Except, therefore, in the case of inhibition by the roots, the nutrition with respect to this element will be greater in the leaves of plants growing on a plot which received this element than in those of a plant growing on a plot that did not. Translating this in terms of the positions of the respective co-ordinate points on the equilateral triangle *at the first sampling date,* we should find that (if for example nitrogen is the particular element under consideration) the co-ordinate point of the plot treated with nitrogen would be displaced nearer to the summit (N = 100 per cent.) than that of the untreated plot.

After this first sampling, consideration must be given to the respective rates of nitrogen nutrition (3), or, translated in terms of trilinear coordinates, to the direction within the triangle which the respective graphs take.

Two possibilities may occur according to the climatic conditions to which the plants are subjected. In the one case, the action of the meteorological factors may be such as to cause the element (nitrogen in the case considered) to play a major rôle relative to phosphoric acid and potash; this result would be reflected by an ascent of the graph towards the summit of the triangle with progressive increase in the maturity of the leaf.

The addition of a fertilizer containing nitrogen, if effective, would act to cause a greater increase of nitrogen in the NPK-unit and would be translated in the trilinear coordinate graphs in an appropriate manner by a displacement towards the summit.

In the second case, it is possible that the meteorological conditions may be such as to cause the element (we are considering nitrogen) in the *unfertilized* soil to play a minor rôle relative to phosphoric acid or potash. This would be translated by a progressive descent of the graph, with increasing maturity of the leaf, from the summit ($N = 100$ per cent.) of the triangle. In this case the addition of a fertilizer element (nitrogen in the case considered) would act to oppose this descent, but would not necessarily cause a reversal nor even operate to nullify the declining tendency completely.

The action of the fertilizer element would result in producing different yields under the two sets of meteorological factors considered; this action would be favorable when the resultant effect is to cause an approach toward the optimum relations with respect to this element, and unfavorable when the effect is to cause a recession from the optimum.

We can apply the reasoning of the preceding paragraphs to ascertain the influence of the two sets of climatic conditions prevailing in the greenhouse on the rôle of nitrogen, phosphoric acid, and potash, respectively, in the unfertilized (composted) soil.

The influence of the climatic factors on the rôle of nitrogen, in both spring and fall, in the *unfertilized* (composted) soil, is such as to cause the respective graphs to descend progressively with maturity of the leaf in each season. In this respect, then, nitrogen plays a relatively secondary rôle under both climatic conditions, since the rate of supply of nitrogen to the plant is insufficient to prevent a rapid descent of the graph from the summit of the triangle with increasing age of the leaf in either season (fig. 3).

The addition of nitrogen fertilizer (plot no. 12L) in the spring has caused an increase in the nitrogen value of the intensity—percentage of ($N + P_2O_5 + K_2O$) in the dried foliage—throughout the whole cycle; but nitrogen additions in the fall have increased *this constituent of the intensity factor* only during the latter part of the cycle and feebly even then.

The effect of nitrogen additions under spring and fall conditions, respectively, on the changes in the physiological ratios, *i.e.*, in the *NPK-units*, with increasing age of the leaf is shown graphically in figures 3 and 4.

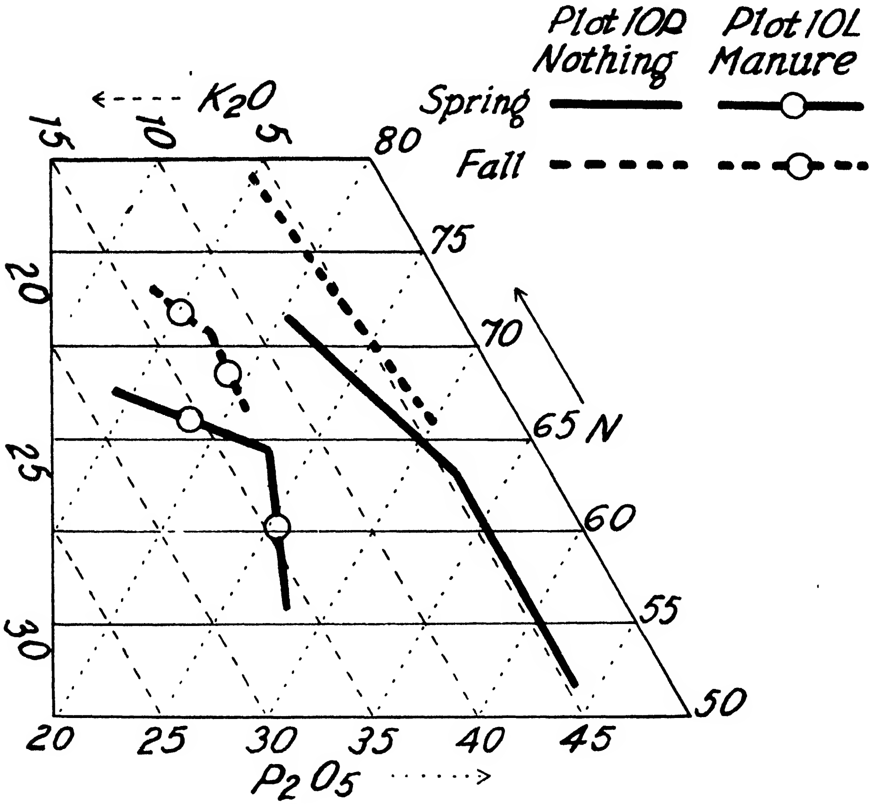

FIG. 3. Changes during the growth cycle in the N–P_2O_5–K_2O equilibrium of the fifth leaf in the spring and also in the fall of plants growing on the manured plot (no. 10L) and unmanured plot (no. 10R) receiving no fertilizer. Only a part of the triangle, the sides of which equal 100 is shown.

Under the climatic conditions prevailing during spring, additions of nitrogen have caused a displacement of the graph towards the summit (N = 100 per cent.); as the relative length of the graphs of the unmanured plots 10R and 12L in the spring show, the added nitrogen has prevented the rapid descent without, however, being able to nullify it.

The addition of nitrogen in the fall has not affected much change in the displacement of the graph from that of the unfertilized plot at this season. Hence in the fall, the effect of added nitrogen on the N–P_2O_5 – K_2O equilib-

rium is almost nil; its effect is not sensibly different from the effect of the nitrogen of the unfertilized soil acting alone in the fall, as the similarity of the forms and positions of the graphs of the unmanured plots no. 12L and no. 10R indicate.

The graphs of the unfertilized plot no. 10R approach progressively (with increasing age of the leaf) towards the right base apex ($P_2O_5 = 100$ per cent.) in the fall as well as in the spring; the rate of increase, however,

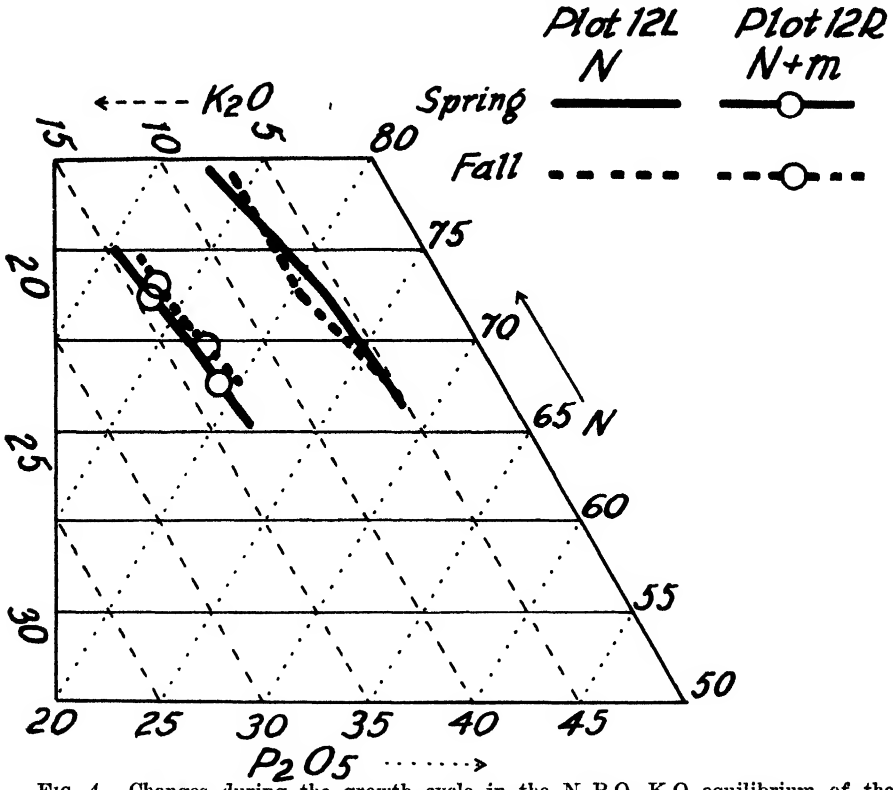

FIG. 4. Changes during the growth cycle in the N–P_2O_5–K_2O equilibrium of the fifth leaf in the spring and fall of plants growing on the manured and unmanured plots receiving nitrogen fertilizer (plots no. 12R and no. 12L, respectively).

is less under the former conditions (fig. 3). The influence, therefore, of the climatic conditions prevailing in the greenhouse on the rôle of phosphoric acid of the unfertilized soil is similar in both spring and fall, and acts in such a manner as to cause this element to play a major rôle relative to nitrogen and potash.

The addition of superphosphate (plot no. 14L) has caused a decrease of this element in the magnitude intensity ($N + P_2O_5 + K_2O$) in the spring and, except during the early portion of the cycle, in the fall also.

The effects of the addition of superphosphate alone, in both the spring and fall, on the quota part of this element in the NPK-unit, with increasing maturity of the leaf, is remarkably small in consideration of the relatively large amounts of superphosphate added (fig. 5). This is shown by the small

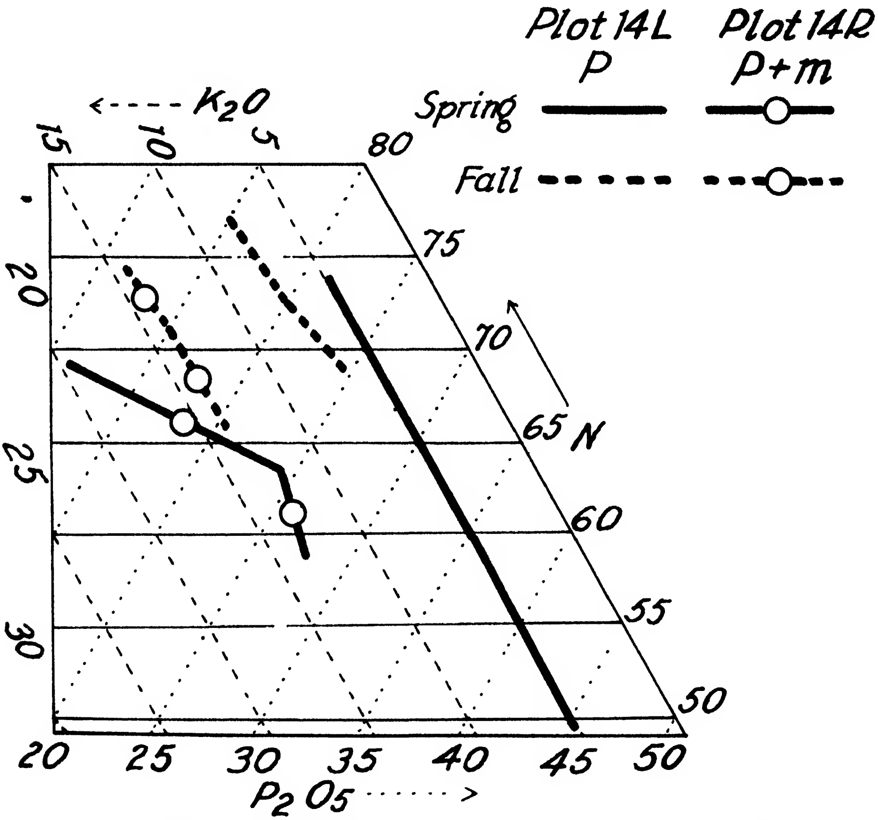

FIG. 5. Changes during the growth cycle in the $N-P_2O_5-K_2O$ equilibrium of the fifth leaf in the spring and also in the fall of plants growing on the manured and unmanured plots receiving superphosphate (plots no. 14R and no. 14L, respectively).

displacement of the graph of 14L towards the right base apex relative to that of 10R. Thus at the first sampling date the values of the quota parts of phosphoric acid of the unfertilized plot and that of the plot which received superphosphate differ only by 1.09 and 0.26 units, in spring and fall, respectively. Under fall conditions the graph of the phosphate plot is much shorter relative to that of the unfertilized soil, indicating relatively lower utilization.

There is no plot receiving only potash in this experiment. Inasmuch as the reciprocal effects between potassium and phosphorus are, in general, not

large (2), the [PK] plot (no. 20R) can be used to examine the effects of meteorological conditions on the action of the element potash.

The graphs of the unfertilized plot (no. 10R) show that during both spring and fall the changes in the displacements of the NPK-units with increasing age of the leaf are small (fig. 3). In both seasons the graphs move progressively away from the left base apex ($K_2O = 100$ per cent.) with increasing age of the leaf.

The addition of potash (plot 20R) has caused an increase in the value of

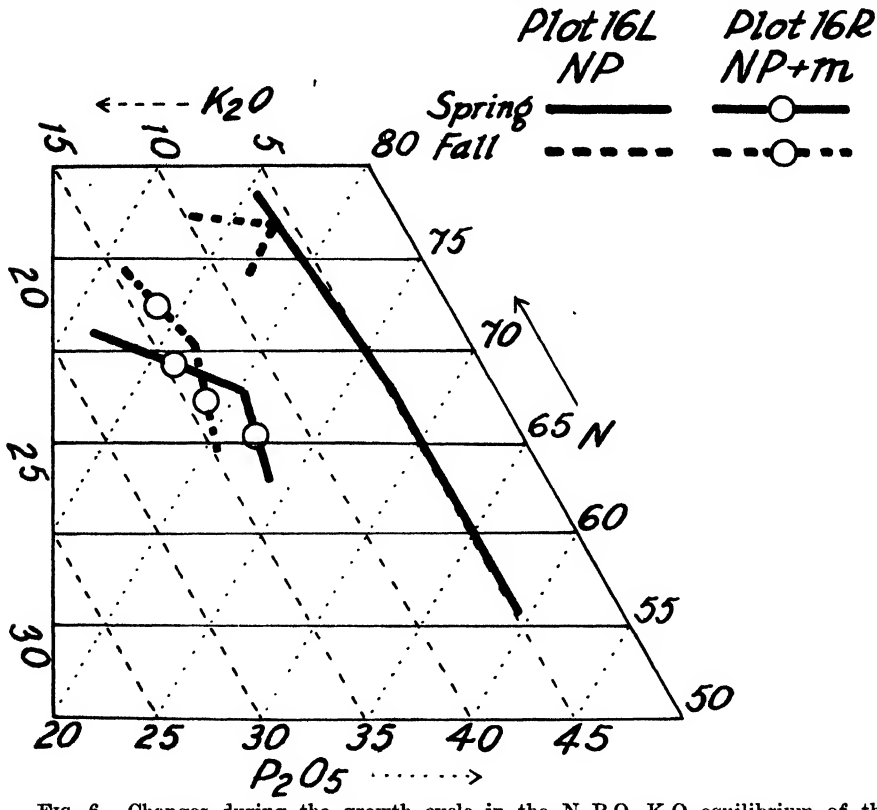

FIG. 6. Changes during the growth cycle in the N–P_2O_5–K_2O equilibrium of the fifth leaf in the spring and also in the fall of plants growing on the manured and unmanured plots receiving nitrogen and superphosphate (plots no. 16R and no. 16L, respectively).

this element in the magnitude intensity ($N + P_2O_5 + K_2O$) throughout the whole cycle in both seasons, and also has produced a marked displacement towards the left base apex ($K_2O = 100$ per cent.) of the graph of the plot which received potash (fig. 8), relative to that of the unfertilized plot (fig.

3). The addition of potash, however, has not been sufficient to nullify the decline with increasing age of the leaf in the quota part of this element.

The mean value of the intensities of nutrition and of the NPK-units resulting from each treatment under spring and fall conditions, respectively.—The mean of the values, at the three sampling dates, of the intensities and of the *NPK-units*, respectively, for each treatment represents the resultant of the values during the growth cycle, and, consequently, simplifies consid-

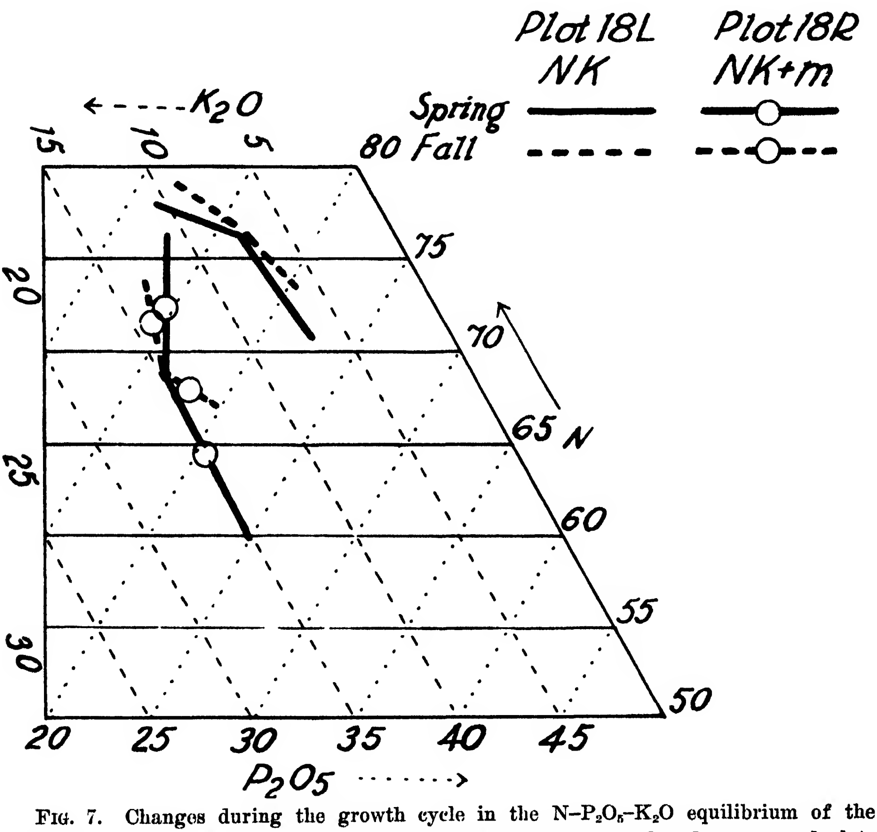

FIG. 7. Changes during the growth cycle in the N–P_2O_5–K_2O equilibrium of the fifth leaf in the spring and fall of plants growing on the manured and unmanured plots receiving nitrogen plus potash (plots no. 18R and no. 18L, respectively).

eration of the relationship of the effects of the treatments under the two seasonal influences.

The mean values of the intensities are given in the fourth column of table III and the mean *NPK-units* in the fifth column; the latter are shown graphically in figures 12 and 13 by means of a point which represents the center of gravity of the corresponding detailed diagrams (figs. 3–11).

TABLE III

THE RELATION OF THE MEAN INTENSITY AND MEAN NPK-UNIT OF THE FIFTH LEAF IN THE SPRING AND FALL TO YIELDS OF PLANTS RECEIVING THE TREATMENTS INDICATED WITHOUT MANURE

SEASON	PLOT	TREATMENT	MEAN INTENSITY	MEAN NPK-UNIT N	MEAN NPK-UNIT P_2O_5	MEAN NPK-UNIT K_2O	YIELD OF FRUIT IN POUNDS
Spring Fall	10R	nothing	6.42 6.61	62.20 72.52	32.06 22.18	5.74 5.30	88.1 54.7
Spring Fall	12L	N	5.89 7.21	72.71 73.05	21.02 20.59	6.27 6.36	64.4 54.6
Spring Fall	14L	P	5.79 7.13	61.07 73.17	33.92 19.45	5.01 7.38	43.3 64.9
Spring Fall	16L	NP	5.79 7.22	67.40 76.07	27.37 15.73	5.23 8.20	30.9 70.2
Spring Fall	18L	NK	5.86 7.21	74.94 76.36	16.80 16.21	8.26 7.43	95.6 67.3
Spring Fall	20L	PK	5.71 7.78	61.95 70.72	27.20 18.86	10.84 10.42	98.8 70.5
Spring Fall	2R	NPK	6.10 7.21	71.04 73.03	21.20 18.63	7.75 8.34	113.8 65.1
Spring Fall	4R	(2N)PK	6.46 7.04	71.58 75.54	20.96 17.92	7.46 6.54	107.3 62.3
Spring Fall	8R	(RN)PK	6.77 7.20	75.37 75.02	16.59 17.77	8.04 7.21	104.5 62.6

TABLE III—(*Concluded*)

THE RELATION OF THE MEAN INTENSITY AND MEAN NPK-UNIT OF THE FIFTH LEAF IN THE SPRING AND FALL TO YIELDS OF PLANTS RECEIVING THE TREATMENTS INDICATED WITH MANURE

SEASON	PLOT	TREATMENT	MEAN INTENSITY	MEAN NPK-UNIT N	MEAN NPK-UNIT P_2O_5	MEAN NPK-UNIT K_2O	YIELD OF FRUIT IN POUNDS
Spring Fall	10L	manure	7.17 8.43	62.58 70.03	21.66 17.13	15.76 12.84	113.2 63.2
Spring Fall	12R	N + manure	7.64 8.56	69.92 71.29	16.31 15.64	13.77 13.07	121.9 60.1
Spring Fall	14R	P + manure	6.58 9.20	63.81 70.56	21.13 15.54	15.06 13.90	113.5 53.6
Spring Fall	16R	NP + manure	7.36 8.95	67.24 69.79	18.53 16.14	14.23 14.07	117.9 71.3
Spring Fall	18R	NK + manure	7.94 8.70	68.33 69.97	18.00 16.37	13.67 13.66	120.5 68.1
Spring Fall	20R	PK + manure	7.85 9.02	62.26 67.25	20.27 16.89	17.47 15.86	129.3 85.2
Spring Fall	2L	NPK + manure	7.54 8.88	68.40 70.39	14.21 14.61	17.39 15.00	121.8 60.7
Spring Fall	4L	(2N)PK + manure	7.34 8.34	69.98 71.04	16.38 15.72	13.64 13.24	115.5 67.1
Spring Fall	8L	(RN)PK + manure	7.83 8.59	71.22 72.55	13.89 15.52	14.89 11.93	120.5 60.9

The resultant intensity of a particular plot is higher in the fall season than in the spring in both the manured and the unmanured series, respectively.

The quota for nitrogen in the NPK-unit is higher in every case in the fall than in the spring, and that for phosphoric acid and potash lower with a few exceptions. In the case of potash the deviation is explicable as the

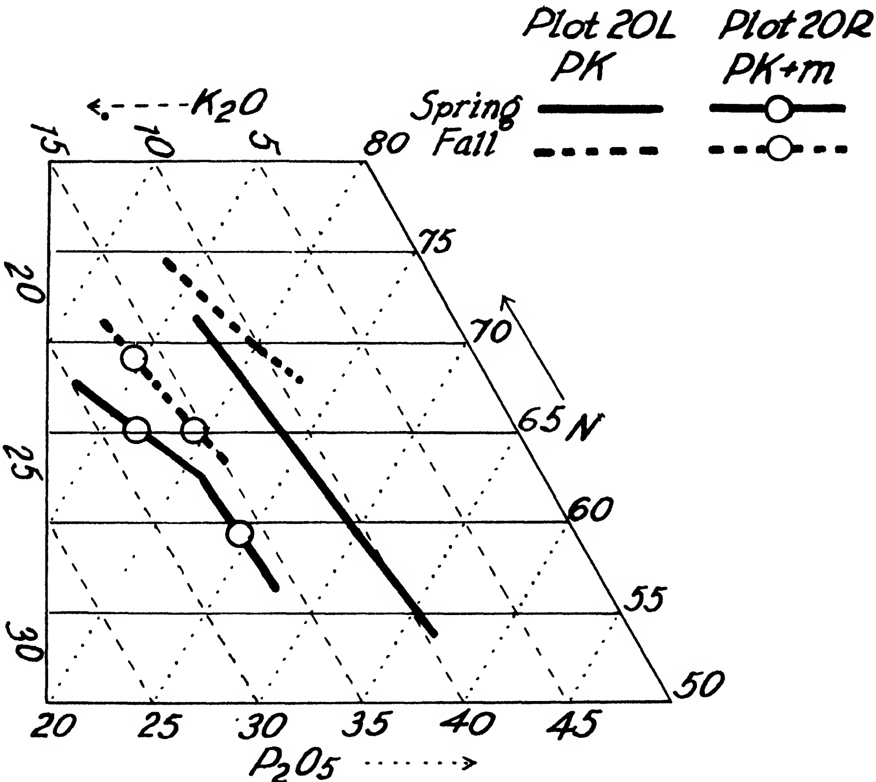

FIG. 8. Changes during the growth cycle in the N–P_2O_5–K_2O equilibrium of the fifth leaf in the spring and fall of plants growing on the manured and unmanured plots receiving phosphate and potash (plots no. 20R and no. 20L, respectively).

result of diseased conditions of the plants from the [N], [P], and [NP] treated plots in the spring (**11, 12**).

Relation of positions on the triangle to yields under spring and fall conditions.—The higher yields in the spring season from a particular treatment are associated in the manured series with a displacement of a co-ordinate point in the spring relative to that in the fall further away from the summit of the triangle (N = 100 per cent.), further towards the left base apex

($K_2O = 100$ per cent.), and (except in two cases) further towards the right base apex also ($P_2O_5 = 100$ per cent.).

In the unmanured series in those plots in which the yield of fruit was higher in the spring season than in the fall the displacement of a co-ordinate point in the spring relative to that of the corresponding co-ordinate point in the fall is further away from the summit ($N = 100$ per cent.), further towards the right base apex ($P_2O_5 = 100$ per cent.) and further towards the left base apex ($K_2O = 100$ per cent.).

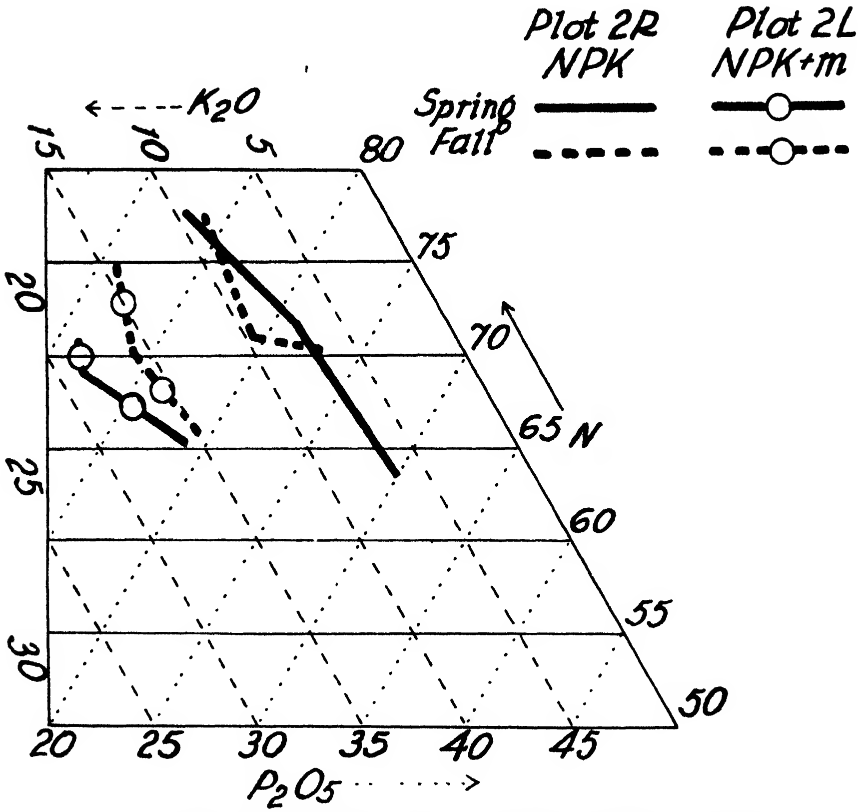

FIG. 9. Changes during the growth cycle in the N–P_2O_5–K_2O equilibrium of the fifth leaf in the spring and fall of plants growing on the manured and unmanured plots receiving nitrogen, phosphate, and potash (plots no. 2L and no. 2R, respectively).

In the two plots in the unmanured series in which a given treatment resulted in a lower yield in the spring than in the fall, namely plots [P] no. 14L and [NP] no. 16L, the lower yield obtained in the spring season is associated with a displacement of the coordinate point in the spring relative to that in the fall away from the summit of the triangle (=100 per cent.)

further towards the right base apex ($P_2O_5 = 100$ per cent.) and further away from the left base apex ($K_2O = 100$ per cent.)

Summary

Tomatoes were grown in the spring and fall, respectively, of the year 1938 under the environmental conditions prevailing in a greenhouse.

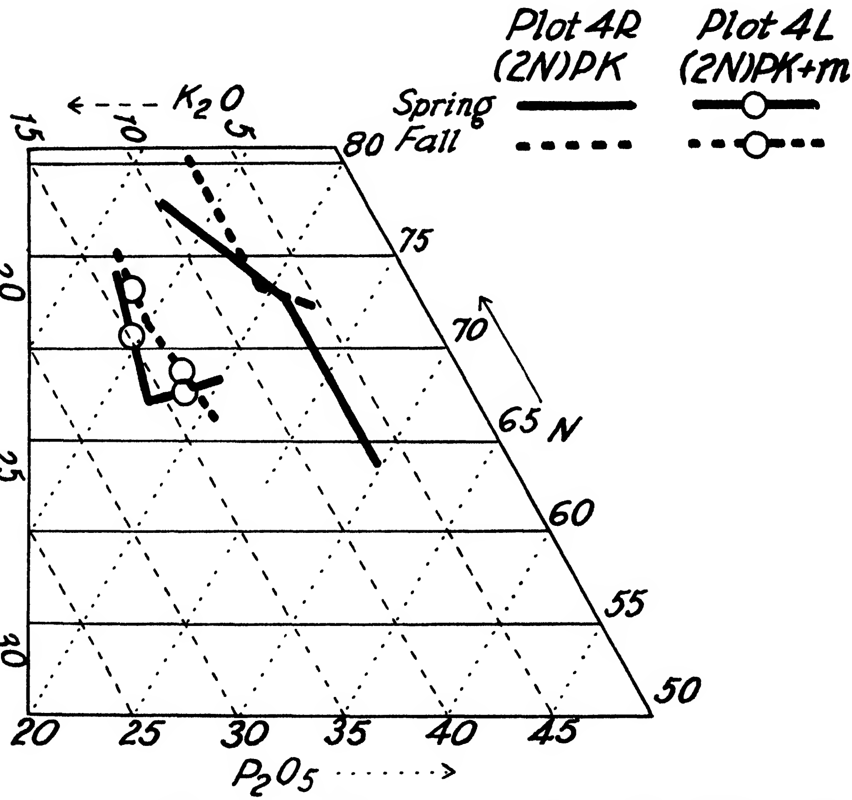

FIG. 10. Changes during the growth cycle in the N–P_2O_5–K_2O equilibrium of the fifth leaf in the spring and fall of plants growing on the manured and unmanured plots receiving twice the unit amount of nitrogen plus phosphate and potash (plots no. 4L and no. 4R, respectively).

The fertilizer treatments in each season consisted of two series: one with manure dressings and the other without. Both series received commercial fertilizer amendments consisting of a single element on certain plots and combinations of two and also of all three elements nitrogen, phosphoric acid, and potash on others.

In the manured series the yield of a particular plot in the spring was in

most cases twice that of the same plot in the fall. And, except in the case of plots receiving the treatments (1) phosphate alone and (2) phosphate plus nitrate without potash (on which the plants growing in the spring were non-resistant to an attack of fusarium wilt of tomato) the yields of the unmanured series were also greater in the spring than those of the same plots, respectively, in the fall.

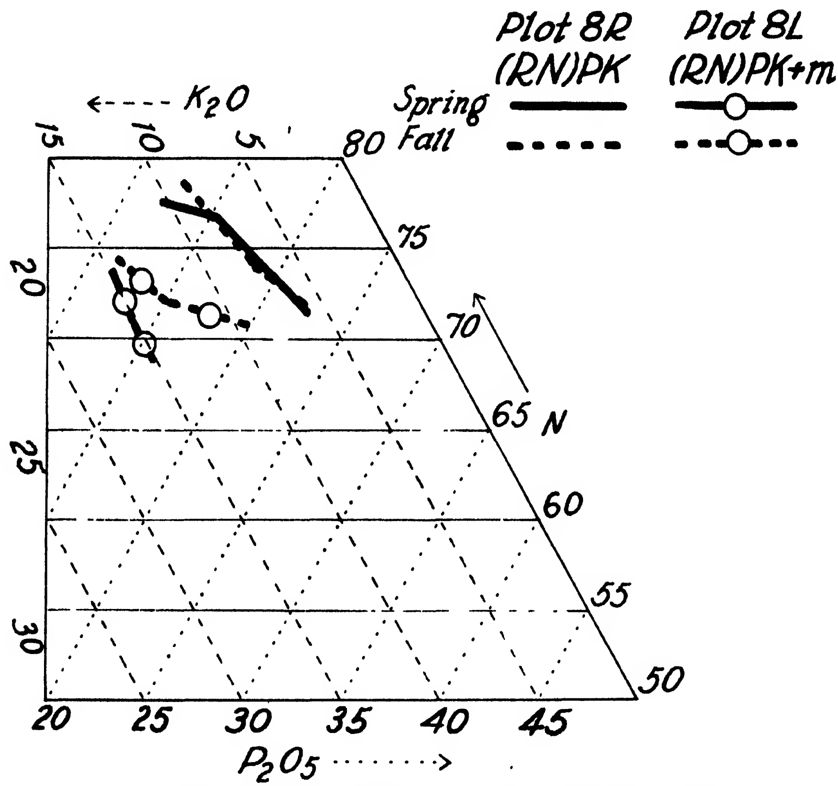

FIG. 11. Changes during the growth cycle in the N–P_2O_5–K_2O equilibrium of the fifth leaf in the spring and fall of plants growing on the manured and unmanured plots receiving biweekly additions of nitrogen together with phosphate and potash (plots no. 8L and no. 8R, respectively).

The foliar diagnosis of the fifth leaf from the base shows the following characteristics, the significance of which is discussed.

The intensity of nutrition resulting from a given treatment is always higher in both series in the fall.

The quota of both nitrogen and potash in the NPK-unit (N–P_2O_5–K_2O equilibrium) decreases progressively with increasing age of the leaf in both series during both spring and fall. The value (quota part) for nitrogen

for a particular plot is higher and that for potash is lower during most of the cycle in the fall than in the spring.

The quota of phosphoric acid in the NPK-unit increases progressively with increasing maturity of the leaf in both series during both seasons. With exceptions noted, the value (quota part) for phosphoric acid in the NPK-unit for a given plot is less in the fall than in the spring during the latter part of the cycle.

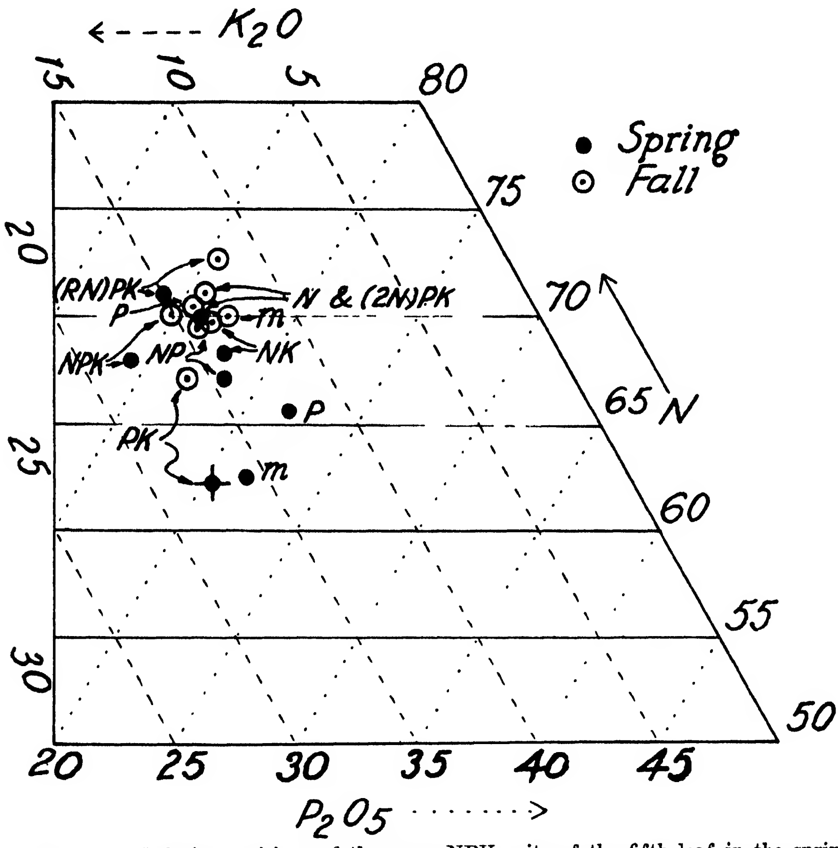

FIG. 12. Relative positions of the mean NPK-units of the fifth leaf in the spring and fall respectively of plants growing on the plots indicated (unmanured series).

The mechanism of the action of a particular fertilizer element in different (meteorological) years or seasons is deduced in terms of the established concepts of foliar diagnosis, and is applied in the present experiment to the examination of the effect of the meteorological conditions prevailing during the spring and fall seasons, respectively, on the rôle of nitrogen, phosphoric acid, and potash in the unfertilized soil.

Whereas, in the spring, the addition of nitrogen has caused an increase of this element in the value of the intensity of nutrition; under fall conditions, the increase occurs only during the latter part of the cycle and is feeble even then. The addition of potash has produced a marked increase in the value of this element in the magnitude intensity in both spring and fall. The ad-

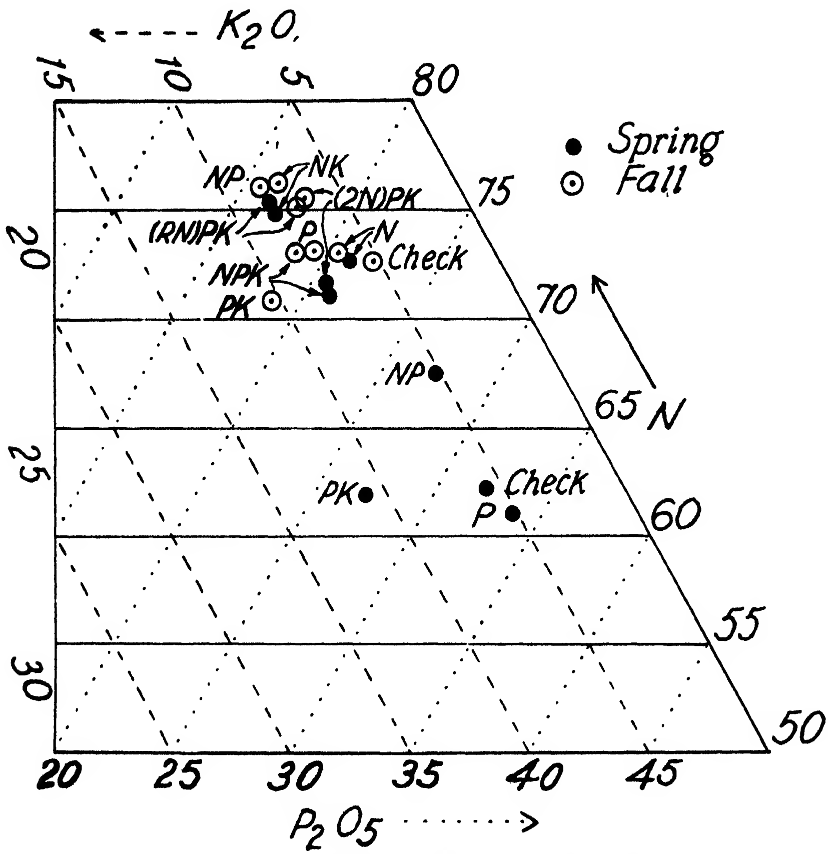

FIG. 13. Relative positions of the mean NPK-units of the fifth leaf in the spring and in the fall respectively of plants growing on the plots indicated (manured series).

dition of phosphoric acid, on the other hand, has led to a decrease of this element in the magnitude intensity during the latter part of the cycle in both seasons.

Compared with the plants growing on the unfertilized plot the addition of nitrogen has caused a marked increase in the quota of nitrogen in the NPK-unit in the spring but has little effect in the fall. Potash additions also have caused a marked increase in the quota of this element in the NPK-

unit in both spring and fall; but the addition of phosphoric acid has had relatively small effect.

The higher yields of a given treatment in the spring compared with that from the same plot in the fall are associated with a lower value for the quota of nitrogen and also for phosphoric acid in the composite NPK-unit, and a higher value for potash.

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EFFECT OF THE FORM OF THE AVAILABLE NITROGEN ON THE CALCIUM DEFICIENCY SYMPTOMS IN THE BEAN PLANT

JOHN SKOK

(WITH THREE FIGURES)

Introduction

Calcium has been found to be one of the most important mineral elements needed for normal plant growth. In its absence plants exhibit very severe deficiency symptoms usually very early and if calcium is withheld further, death always results. NIGHTINGALE *et. al.* (7) made a detailed study of the responses of the tomato plant to calcium deficiency. The reason that calcium deficiency has a more severe effect on a plant than the deficiency of almost any other single element is probably in part because calcium has been found to have many functions in growth and development. These various rôles of calcium will not be discussed here, with the exception of one: the effect of calcium on nitrogen metabolism, as it is directly concerned with this report.

After ECKERSON (2) devised a method for measuring reductase activity, the plant's capacity to reduce nitrates to nitrites, which is the first phase of protein synthesis, she later studied the conditions which affect nitrate reduction and found calcium among other things to be essential (3). Other workers have also found that calcium malnutrition impairs normal nitrogen metabolism. BURRELL (1) found soybeans grown without calcium to accumulate nitrates in the leaves, and to have a much smaller amount of insoluble and amino acid nitrogen than plants grown with calcium; he believes this condition is brought about by reduced nitrate reduction. GINSBURG and SHIVE (5) on the other hand, also working with the soybean believe that calcium has no effect on the protein content. HIBBARD and GRIGSBY (6) found calcium-deficient peas to contain smaller amounts of protein than the control plants but believe this is caused by the general disturbance of the plants rather than by the lack of any particular element. Presenting calcium and nitrogen analyses of a large number of plants PARKER and TRUOG (8) found a very close correlation between the calcium and nitrogen content.

If minus calcium plants lose their capacity to reduce nitrates and synthesize proteins, they are essentially minus nitrogen as well as minus calcium. The typical calcium deficiency symptoms then must be caused by both of these factors.

The question as to just what symptoms would develop with calcium deficiency if nitrate reduction were made unnecessary, prompted these

experiments in which a reduced form of nitrogen was supplied to the plants, and compared to those receiving oxidized nitrogen.

Materials and methods

The plant used in this study was the Dwarf Red Kidney bean, *Phaseolus vulgaris*. The seeds were planted in flats of unused quartz sand receiving only distilled water and allowed to germinate and grow in the greenhouse. After about 7 days plants of uniform size were transplanted to 1.5-liter glazed earthenware pots filled with unused quartz sand. The holes in the center of the pots were covered with small amounts of glass wool to insure good drainage, and to keep the sand from washing through. Two plants were planted in each pot. One day after transplanting (8 to 9 days after planting the seeds) the plants received the first application of nutrients, and every second day thereafter. Merck's reagent quality chemicals were used for all solutions. The reduced forms of nitrogen used in the first preliminary experiments were urea, and ammonia in the form of NH_4Cl. Ammonia proved to be very toxic to the bean plant, causing severe burning of the leaves and eventual death. This symptom was clearly evident within a week after the first application. Concentrations of NH_4Cl from 0.015 to 0.0045 M were tested, and the nutrient solutions were run at pH 4.7 and 6.0. Since all of these solutions containing ammonia proved to be very toxic, the remainder of the experiments were devoted to the use of urea as compared to NO_3 nitrogen.

Only a few references can be found in the literature concerning the use of urea in growing plants. PIRSCHLE (9) grew several different plants in water culture supplying urea to some and expresses the opinion that urea can be changed to ammonia inside the plant independently of bacterial action. YAMAGUCHI (10) grew corn seedlings in sterile culture with urea as a source of nitrogen. He reports that urea was absorbed and was a readily available source of nitrogen for plant growth.

The following groups of plants were grown in each experiment: (1) $+Ca$, $+NO_3$; (2) $-Ca$, $+NO_3$; (3) $+Ca$, $+CO(NH_2)_2$; and (4) $-Ca$, $+CO(NH_2)_2$. Twenty-eight to 30 plants were grown under each nutrient in each experiment. The minus-calcium plants received no calcium whatsoever at any time during their growth.

Various concentrations of the salts making up the nutrient solutions were tried and one of the more dilute ones was finally chosen. An attempt was made to keep the $MgSO_4$ and KH_2PO_4 rather low so that they would not become excessively toxic in the absence of calcium and over-emphasize the true calcium deficiency symptoms and yet not have them too low to be limiting for best growth. GAUCH (4) found magnesium to be very toxic in the absence of calcium, and by lowering it considerably the $-Ca$ plants were

able to live longer, take up more NO_3 and other minerals and synthesize more materials including nitrogen compounds.

The composition of the nutrient solutions used is given in table I.

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS*

Solution	Reaction of solution	Molar concentration					
		$Ca(NO_3)_2$	$NaNO_3$	$CO(NH_2)_2$	$CaCl_2$	KH_2PO_4	$MgSO_4$
	pH						
+ Ca + NO_3	4.7	0.0045				0.0025	0.001
− Ca + NO_3	4.7		0.0045			0.0025	0.001
+ Ca + $CO(NH_2)_2$	4.9			0.0045	0.0045	0.0025	0.001
− Ca + $CO(NH_2)_2$	5.1			0.0045		0.0025	0.001

* All solutions also contain the following micro nutrients: 0.5 p.p.m. B as H_3BO_3; 0.5 p.p.m. Fe as ferric citrate; 0.5 p.p.m. Mn as $MnCl_2$; 0.5 p.p.m. Zn as $ZnCl_2$; and 0.125 p.p.m. Cu as $CuCl_2$.

Experimentation

The experimental data here presented consists of a report of but two experiments, as the preliminary experiments conducted gave essentially the same results.

Experiment I was started in June, 1939, and the plants were harvested 30 days after planting, about the time flower buds began to appear. Experiment II was set up in September, 1939, and the plants were allowed to grow to maturity, 52 days after planting. Wet and dry weights were taken of all plants, and photographs were taken of the plants in experiment II at the time of maturity and at the age of 30 days. Notes were kept on the appearance of the plants in the course of the experiment. No chemical analyses were made at this time.

EXPERIMENT I.—About 15 days after time of planting, the − Ca, + NO_3 plants showed definite symptoms. The plants were 12 to 15 cm. tall and had less leaves than the + Ca plants. The old leaves were dark and the young leaves very chlorotic at this time. The + Ca, + NO_3 plants were 15 to 20 cm. tall, had more and larger leaves than the former group, and were grass green in color. The + Ca, + $CO(NH_2)_2$ plants were 12 to 15 cm. tall, and were darker green in color than the + Ca, + NO_3 group. They had also fewer new leaves than this latter group; some of the older leaves were slightly yellow, and in some cases very slightly burned at the margins only. The − Ca, + $CO(NH_2)_2$ plants at this time were 12 to 15 cm.

tall, had fewer leaves than the $+Ca$, $+NO_3$ group, and the new leaves were a little smaller. The plants on the whole had a very good appearance and were exceptionally dark green. They had the darkest green color of the entire group. The new leaves also were very dark green (the new leaves are usually lighter than the old leaves). There was no burning of leaves nor injury of any kind at this time. The contrast of this group with the $-Ca$, $+NO_3$ group was very striking.

Fifteen days later (30 days after planting) this series of plants was harvested. At this time flower buds had formed on all plants except the $-Ca$, $+NO_3$ group. The $+Ca$, $+NO_3$ plants were very vigorous, green and had a good development of roots which were yellowish brown in color. The $-Ca$, $+NO_3$ plants showed considerable injury. They were very chlorotic and the tips were completely dead. The roots were poorly developed and had a dark brown color. The epidermis was loose and had been lost from most of the roots. The $+Ca$, $+CO(NH_2)_2$ plants were smaller than the $+Ca$, $+NO_3$ plants and not as vigorous. The leaves were paler green in color and some of the lower leaves were chlorotic. The roots were fairly well developed and very light in color, in fact the lightest of all four groups. The $-Ca$, $+CO(NH_2)_2$ plants were still dark green in color, although the lower leaves were somewhat burned and their tips beginning to die. The upper parts of the plants were in very good condition with no injury to the growing tips. The roots were fairly well developed and had a yellowish brown color and no loose epidermis. They were lighter in color than those of the $+Ca$, $+NO_3$ group and slightly darker than the $+Ca$, $+CO(NH_2)_2$ group. The appearance of both the top portion and the roots of the $-Ca$, $+CO(NH_2)_2$ group was decidely not characteristic of the usual $-Ca$ symptoms.

Data on the plants of experiment I are presented in table II.

EXPERIMENT II.—The plants in this experiment were grown under the same conditions as those in experiment I, but they were grown to maturity. The following description applies to plants at 19 days of age. The $+Ca$, $+NO_3$ plants were 25 to 27 cm. tall, with large green leaves. The new leaves were lighter than the old leaves. The $+Ca$, $+CO(NH_2)_2$ plants were 23 to 26 cm. tall and were about like those in the former group except that the new leaves were not lighter than the old leaves. The $-Ca$, $+NO_3$ plants were 21 to 27 cm. tall, and the upper leaves were rather pale (paler than those of the $+Ca$, $+NO_3$ group) and some had grayish spots. The plants in general were almost as tall as the $+Ca$, $+NO_3$ group but were not as vigorous and had not attained as much growth. The $-Ca$, $+CO(NH_2)_2$ group was 24 to 27 cm. tall and very good in appearance. The plants had a dark green color; none of the leaves, including the new leaves, were light. The leaves were slightly smaller than those of the $+Ca$, $+NO_3$ group, but otherwise growth was about equal in both groups.

TABLE II

DATA OF EXPERIMENT I, PLANTS AT 30 DAYS

WEIGHTS ON 30-PLANT BASIS

Nutrient	Average height	Wet top weight	Wet root weight	Dry top weight	Dry root weight	Percentage dry weight entire plant	Percentage dry weight tops	Percentage dry weight roots	Wet top-root ratio	Dry top-root ratio
	cm.	gm.	gm.	gm.	gm.	%	%	%		
+ Ca + NO_3	55	596.50	195.21	72.15	19.85	11.62	12.09	10.16	3.05	3.63
− Ca + NO_3	15	100.20	42.30	17.20	3.75	14.70	17.16	8.86	2.36	4.58
+ Ca + $CO(NH_2)_2$	40	344.06	97.96	36.51	8.30	10.13	10.61	8.47	3.51	4.40
− Ca + $CO(NH_2)_2$	20	170.62	76.59	26.72	6.53	13.45	15.66	8.52	2.22	4.16

Thirteen days later (31 days after planting) photographs were taken of the plants in this experiment which are shown in figure 1.

Following is a description of the plants at this date. The + Ca, + NO_3 plants were 40 to 60 cm. tall, had large green leaves, and many flower buds. The + Ca, + $CO(NH_2)_2$ plants were 40 to 50 cm. tall, and the leaves were smaller than those of the preceeding group. The leaves were also decidedly mottled, somewhat resembling either iron or magnesium deficiency. Many flower buds were present. The − Ca, + NO_3 plants were 25 to 30 cm. tall and very poorly developed. The upper leaves were yellow, with gray and brown necrotic spots. All the leaves were small and a few poorly developed flower buds were present. The − Ca, + $CO(NH_2)_2$ plants were 30 to 45 cm. tall and their general appearance was rather good. Their color was still fairly dark green and many flower buds were present. Some leaves, however, at this time had become mottled with very small yellowish, grayish, to brownish spots. The veins, particularly, of some leaves were becoming discolored. The stems of some plants also showed small brown spots. Many of the younger leaves showed their first injury and discoloration at the veins and pulvinus region. This response was also observed by GAUCH (4). Most of the flower buds were rather well developed.

Four days later (35 days after planting) the − Ca symptoms of the − Ca, + $CO(NH_2)_2$ plants, although much delayed as compared to that of the − Ca, + NO_3 plants, were becoming very evident and rather severe. The growing tips of a few plants were dead or dying and several of the leaves had become pale and developed grayish or brownish spots. The plants, however, were still rather green with many uninjured flowers and in decidedly better condition than the − Ca, + NO_3 plants.

Nine days later (44 days after planting) the following observations were made: The + Ca, + NO_3 plants had all set fruit, and were in good condition. The + Ca, + $CO(NH_2)_2$ plants had by this time developed very mottled and chlorotic leaves but the plants otherwise were fairly sturdy in appearance and were bearing fruit in about the same abundance as the plants in the + Ca, + NO_3 group. The upper portions of the − Ca, + NO_3 plants were all dead at this time and all their leaves were rather chlorotic. Some of the plants had flowered but none produced fruit. The − Ca, + $CO(NH_2)_2$ plants were in a much less severe condition. The very uppermost portion of the tips of slightly over half of the plants in this group were dead and the upper leaves were yellowed, some having grayish brown spots. The other leaves still had a rather dark green color, even though some of these same leaves had small spots. All the plants of this group had flowered, and several were bearing fruit.

Eight days later (52 days after planting), these plants were harvested. Photographs were again taken at this time and are shown in figure 2.

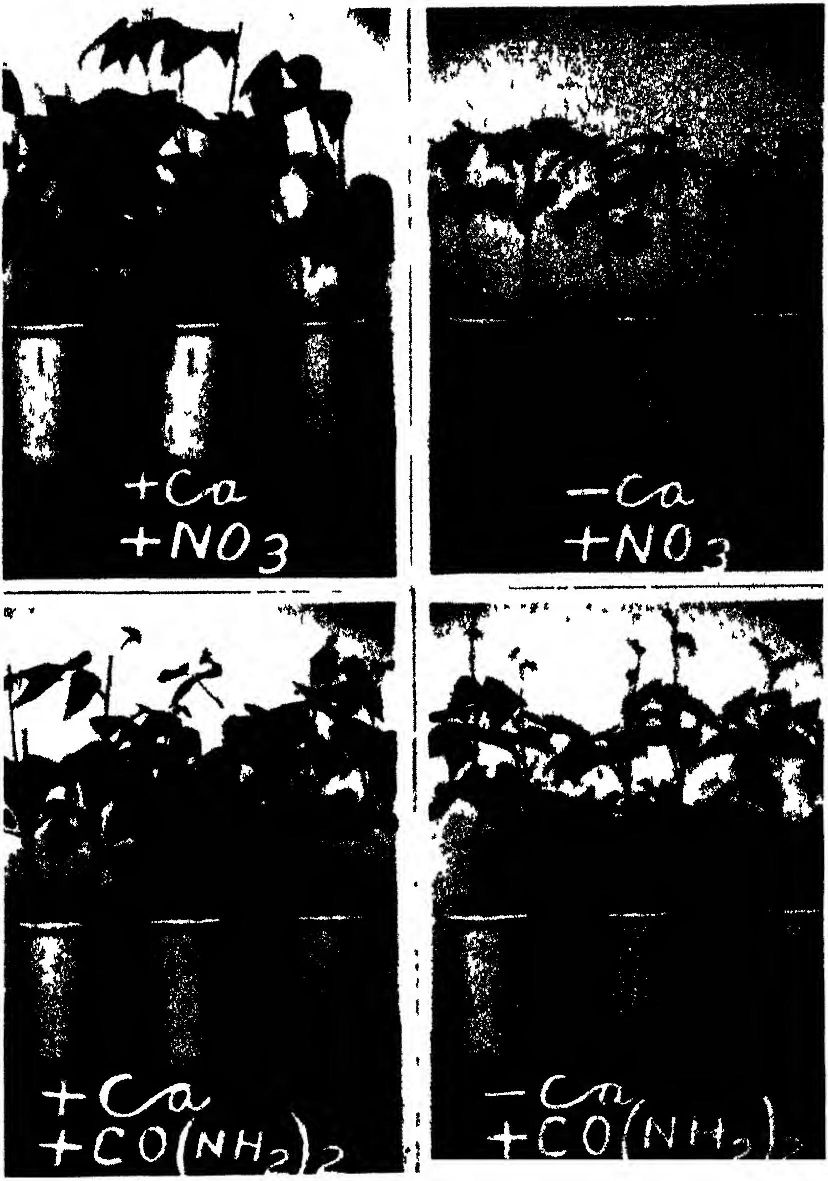

FIG. 1. Experiment II Plants at 31 days.

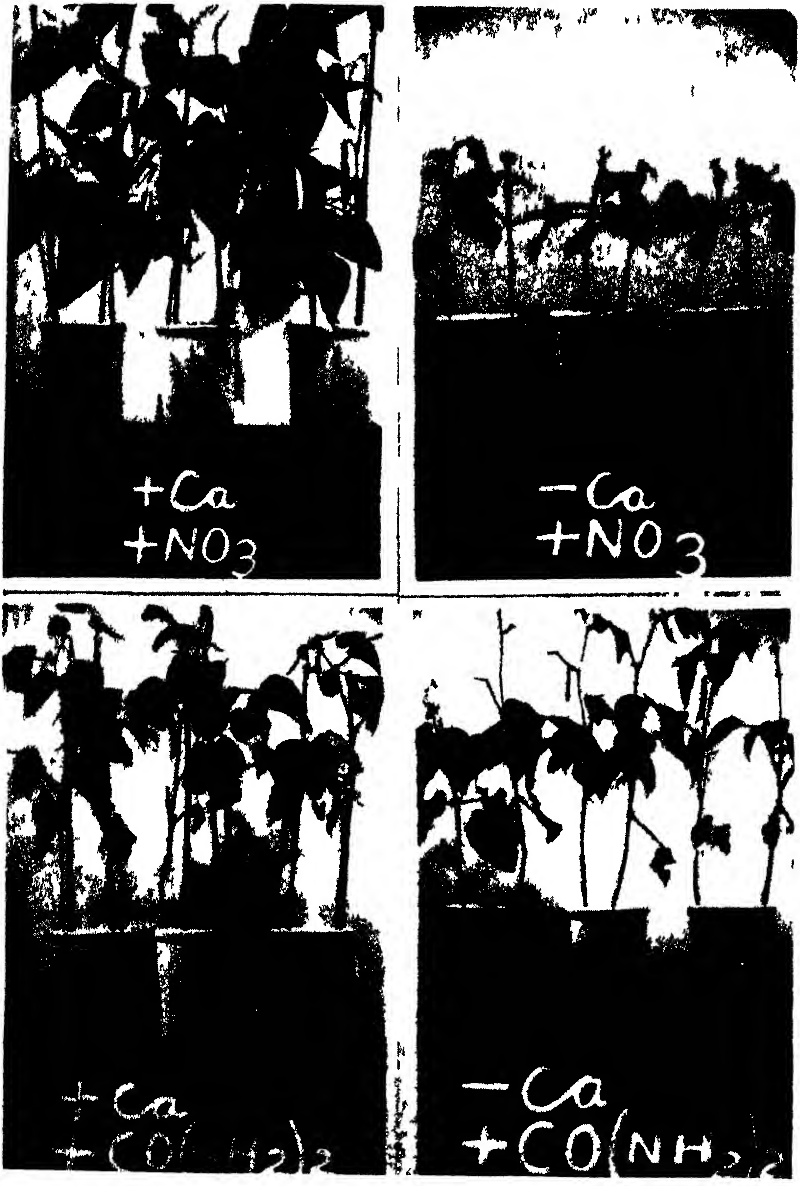

FIG 2. Experiment II. Plants at maturity (52 days).

A brief description of the plants at this date follows: The + Ca, +NO_3 plants were about 60 cm. tall, had large leaves and bore many beans on each plant. The roots were well developed, and light brown in color. The leaves of the + Ca, + $CO(NH_2)_2$ plants were very chlorotic and smaller than those in the preceding group, and most of the lower leaves were dead. All of the plants produced fruit. The roots were well developed, and yellowish brown in color. They were just slightly lighter in color than those of the + Ca, + NO_3 plants. The – Ca, + NO_3 plants were very badly injured. The tops of all plants were dead and all the leaves, even the lowermost, were partially injured. None of the plants produced fruit. The roots were very poorly developed, and dark brown in color. The epidermis of the roots was loose, giving the roots a slimy texture. The base of the stem (lower hypocotyl) was also dark brown and necrotic. The – Ca, + $CO(NH_2)_2$ plants were much larger and in not nearly as severe condition as those deficient in calcium but receiving nitrate. The leaves had spots showing injury, but they still retained their dark, almost blue-green color. The tips of almost half the plants were still living. Fruit was produced by 25 per cent. of the plants in this group (8 out of 32 plants). The fruit was small in most cases (largest bean pod produced was 7 cm. long) and few per plant, but not entirely absent as in the case of the – Ca, + NO_3 plants. The roots were fairly well developed. There was considerably better growth than that produced by the – Ca, + NO_3 plants, but less than that produced by either of the + Ca groups. They were darker in color than those of the + Ca plants but considerably lighter than those of the – Ca, + NO_3 plants. The epidermis seemed in good condition and no slimy texture was evident. There were no necrotic areas at the base of the stem.

Table III contains the data of experiment II.

Discussion

From the results obtained in these experiments two things are evident: (1) Under normal conditions including the presence of calcium, urea is not as good a source of nitrogen as is the nitrate form for growth of the bean plant. (2) In the absence of calcium much better growth is made by the bean plant with urea than with nitrates.

It was not determined what factors were responsible in producing poorer growth with urea in the presence of calcium than with nitrate The reactions of the two solutions did not differ greatly, as is recorded in table I. The form of the available nitrogen, however, has a very pronounced effect on the calcium deficiency symptoms. With urea, the calcium deficiency symptoms are much delayed and when they become evident they are very much less severe. The weights of the – Ca, + $CO(NH_2)_2$ plants were about 60 per cent. greater than those of the – Ca, + NO_3 plants. The former plants pro-

TABLE III

Data of experiment II, plants at maturity (52 days)

Weights on 30-plant basis

Nutrient	Average height	Wet* top weight	Wet root weight	Wet fruit weight	Dry* top weight	Dry root weight	Dry fruit weight	Percentage dry weight entire plant	Percentage* dry weight tops	Percentage dry weight roots	Percentage dry weight fruit	Wet† top-root ratio	Dry† top-roo[t] ratio
	cm.	gm.	gm.	gm.	gm.	gm.	gm.	%	%	%	%		
+Ca +NO_3	60	463.50	174.06	307.70	83.05	18.65	22.86	13.18	17.92	10.71	7.56	4.43	5.68
−Ca +NO_3	29	137.93	48.16	None	24.38	5.11		15.84	17.68	10.61		2.86	4.77
+Ca +$CO(NH_2)_2$	53	237.23	98.03	62.39	44.16	8.67	4.71	14.47	18.61	8.84	7.55	3.06	4.91
−Ca +$CO(NH_2)_2$	43	183.05	76.45	4.85	39.84	8.91	0.51	18.63	21.76	11.65	10.52	2.46	4.53

* Fruit not included.
† Fruit included.

duced fruit while the latter did not. Flowering and fruiting is very striking for a minus-calcium plant. Equally striking was the good condition of the roots in the calcium deficient plants receiving urea.

These experiments then may be considered as additional evidence that calcium has an important function in the utilization of nitrogen. The evidence is in agreement with ECKERSON's findings that calcium deficient plants have a lowered reductase activity since the calcium deficient plants receiving urea, which is a reduced form of nitrogen, make much better growth than do those receiving nitrates. The calcium-deficiency symptoms of the plants receiving urea, then, are really truer symptoms which can be directly assigned to the lack of calcium.

Since other elements, namely potassium, phosphorus, and sulphur, were also found to be necessary for normal reductase activity by ECKERSON (**3**), it may be entirely possibly that their deficiency symptoms may also be lessened in severity with the use of urea.

Calcium, of course, is also a building element and therefore cannot be eliminated entirely and still retain good growth. From evidence of an early preliminary experiment it seems that plants receiving urea may be able to carry on good growth with only small amounts of calcium. The plants in this preliminary experiment were grown in used sand which evidently contained small amounts of calcium, (the actual amount being unknown). The $-Ca$, $+CO(NH_2)_2$ plants grown to maturity produced almost as much fruit as the $+Ca$, $+NO_3$ plants. This fact together with the results obtained in experiments I and II points to the possibility that with urea the usual amount of calcium used may be in excess and it may be the factor which causes poorer growth with a $+Ca$, $+CO(NH_2)_2$ solution than with a $+Ca$,

FIG. 3. Plants grown in used sand at 39 days.

$+NO_3$ solution. The plants shown in figure 3 were grown in used sand. They were photographed 39 days after planting. When they were 45 days old, the $-Ca$, $+CO(NH_2)_2$ plants had produced 25 per cent. more total growth than the $+Ca$, $+CO(NH_2)_2$ plants. At maturity (53 days after planting) those receiving no calcium had produced 2.7 times as much fruit as those to which calcium had been added.

More conclusive experiments are needed to elucidate the relationship of calcium to nitrogen metabolism. The minimum amount of calcium required for normal growth with nitrate and with urea should be known. The determination of the reductase activity of plus- and minus-calcium plants grown with urea and with nitrate might throw some light upon its relation to protein synthesis in the two different nitrogen series. Determinations of assimilated nitrogen also should be made for actual comparisons of protein synthesis. Finally, histological examinations would show the condition of the cell wall in each group and aid in determining the minimum amount of calcium needed for normal cell wall development.

Summary

1. Calcium is known to be one of the most important mineral elements needed for normal plant growth. Among other things it is closely associated with nitrogen metabolism. ECKERSON found that it is needed for normal nitrate reduction in the synthesis of proteins. In these experiments the bean plant was grown with and without calcium using nitrate nitrogen and urea, a reduced form of nitrogen.

2. With calcium present nitrate nitrogen produced better growth than urea.

3. In the absence of calcium much better growth was made with urea than with nitrates. With urea the calcium deficiency symptoms were much delayed, and when they became evident they were very much less severe.

4. The calcium-deficient urea plants produced about 60 per cent. more growth than the calcium-deficient nitrate plants and all of them flowered. Twenty-five per cent. of the calcium-deficient urea plants produced fruit, whereas none of the calcium-deficient nitrate plants produced fruit, and only a few flowered. The roots of the calcium-deficient urea plants did not exhibit the usual calcium-deficiency symptoms and they produced twice as much growth as those of the calcium-deficient nitrate plants.

The writer wishes to express his sincere thanks and appreciation to Professor C. A. SHULL for his interest and valuable suggestions made during the course of this work.

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ROOT MODIFICATIONS INDUCED IN *VICIA FABA* BY IRRADIATING DRY SEEDS WITH SOFT X-RAYS

G. F. SMITH AND H. KERSTEN

(WITH TEN FIGURES)

Introduction

In a recent paper concerned with plants grown from dry or unsoaked seeds which had been irradiated with soft x-rays, LONG and KERSTEN (**1**), described the occurrence of structural changes in the leaf tissue. Similar results had previously been reported by NOGUCHI (**2**), who used germinated seeds and x-rays of a shorter wavelength. This paper is a continuation of the study of plants germinated from unsoaked seeds which had been irradiated with soft x-rays; it describes some modifications which occur in the structure and development of their roots.

Methods

The radiation was produced by the same apparatus as that used by LONG and KERSTEN (**1**). It has an approximate intensity-wavelength curve as shown in the upper part of figure 1, and a position in the electromagnetic spectrum as indicated by the arrow in the lower part of figure 1.

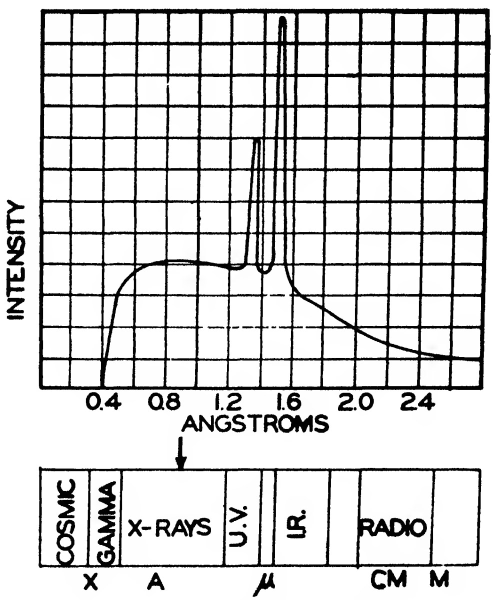

FIG. 1. Upper part: Approximate intensity-wavelength curve for the radiation used. Lower part: General electromagnetic spectrum, with the position of the soft x-rays used, indicated by the arrow.

Unsoaked seeds of *Vicia faba* (Burpee's bush Lima beans) were irradiated by arranging them with their hilum sides 8 cm. from the focal spot of the x-ray tube. Preliminary tests were made to determine a dose of radiation which would produce pronounced root modifications. The treatment selected was one which, after an interval of growth, resulted in the phenomenon of "delayed killing" described by MAXWELL and KEMPTON (3). This employed an exposure of 20 minutes while the tube was operated at 30 peak kv. and 10 ma. The irradiated seeds, with control seeds which had not been irradiated, were germinated in moist peat moss at 85° F. External developments were observed for 8 days, at the end of which time material was prepared for microscopical examination. The following observations, which were made with plants germinated from January 29th to February 6th, using seeds grown during the season of 1939, are in accord with identical experiments carried out during a 3-month period in the fall of 1939.

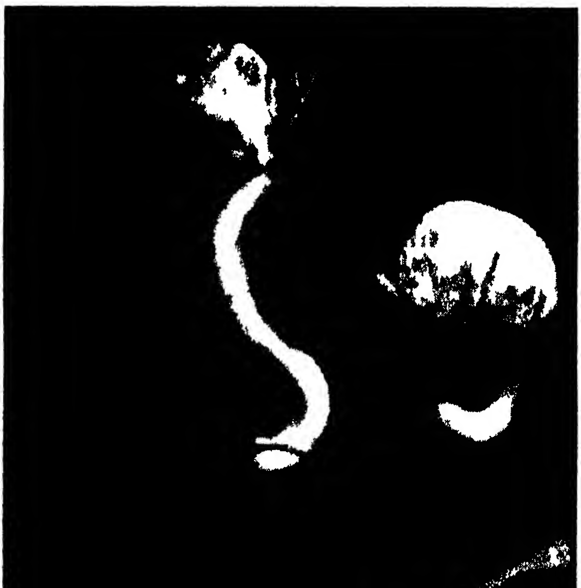

FIG. 2. A normal seedling of *Vicia faba*, and one grown from x irradiated seed. Each seedling is 8 days old. The inset shows an enlarged view of a typical root grown from an irradiated seed.

Gross observations

During the first several days of growth there appear to be no external differences between control and irradiated seeds. The radicles appear at about the same time and continue development at a similar rate for several days. The group germinated from the irradiated seeds later shows a decreased root length, a poor development of the root tip, and the complete absence of lateral roots. By the eighth day, the condition of arrested development appears in the roots of the rayed group. At this time the average length of the primary root of the control plant, including the hypocotyl, is approximately 120 mm., and that of the rayed is approximately 44 mm. (fig. 2).

The inset in figure 2 shows an enlarged view of a typical root of the latter. The bulbous increase in diameter represents the beginning of the hypocotyl which occurs approximately 70 mm. from the tip.

An additional x-irradiation effect is seen when germinating control seedlings, and seedlings grown from x-irradiated seeds, (with combined growth of radicals and hypocotyls 12 to 15 mm.) are marked in mm. lengths. Observations 12 hours after marking show the results illustrated in figure 3.

FIG. 3. Elongation in the primary root and the hypocotyl regions of a control plant, left, and in one germinated from an x-irradiated seed, right.

In the rayed group elongation does not occur within the first originally marked mm. of the primary root, as in the control. This suggests an inactivation of the root tip meristem soon after the radical appears. It is also seen that the elongation of the control hypocotyl is considerably greater than that of the germinating irradiated seed.

Microscopical observations

Material for a microscopical examination was collected from a great number of primary roots of germinating seeds which had been irradiated

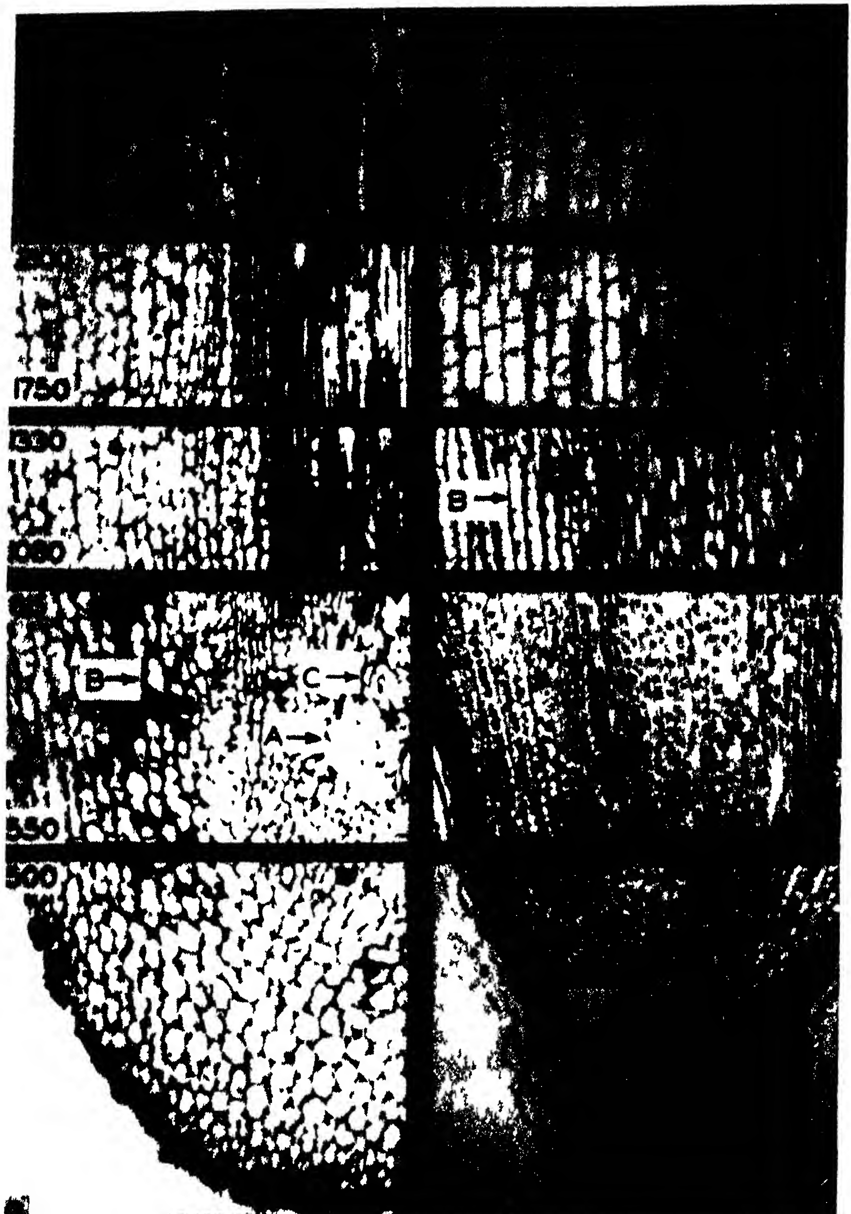

FIG. 4. Longitudinal sections of primary root tips of a normal seedling of *Vicia faba*, right, and one which had been germinated from an x irradiated seed. Generative meristem, A; elongation, B; vascular cells, C. The numbers at the left show the distances from the tip in microns. × about 200.

while unsoaked. This was done 8 days after irradiation when the arrested growth character became evident in the roots. Comparative studies were made with the primary roots of control plants of the same age. Paraffin sections were made and stained with iron-alum haematoxylin and gentian violet.

Examination of the root tip tissues shows many modifications in the case of plants germinated from the x-irradiated seeds as compared with the primary roots of control plants (fig. 4). In seedlings grown from the x-irradiated seeds, the root meristem begins approximately 0.63 mm. from the root tip, while in the primary root of the control, the meristem is approximately 0.225 mm. from the tip. In the root tip of a plant grown from an x-irradiated seed, vascular cells appear approximately 0.70 mm. from the tip, while in the primary roots of the control plants, the first vascular cells occur approximately 3.25 mm. from the root tip. In the rayed group, an obviously peculiar situation is found in the first vascular cells. In many cases these cells possess a typically scalariform type of wall thickening; in others, the reticulate type may occur. This is a modification of the normal condition in *Vicia faba,* where the annular and spiral vascular elements extend for a considerable distance before the scalariform and the reticulate vessels of the metaxylem appear.

Elongation of the cells derived from the root tip meristem, as in the elongation region of normal roots, is but slightly indicated in the roots of seedlings grown from the x-irradiated seeds. In the latter, some suggestion of increased cell size has been observed approximately 0.675 mm. from the

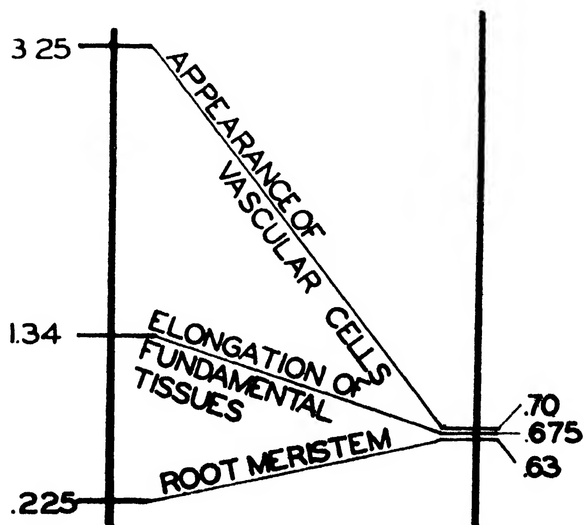

FIG. 5. Diagram of tissue developments in root tips of control seedlings and in those germinated from x-irradiated seeds.

tip, while in the controls, cell elongation is evident approximately 1.34 mm. from the tip. These observations are represented graphically in figure 5 and are listed in table I. Thus the condition found in the irradiated group

TABLE I

AVERAGE CELL LENGTHS AT VARIOUS DISTANCES FROM THE TIPS OF *VICIA FABA* SEEDLINGS GROWN FROM X-IRRADIATED SEEDS AS COMPARED WITH CONTROLS

DISTANCE FROM TIP	AVERAGE CELL LENGTH	
	CONTROL	IRRADIATED
mm.	μ	μ
0–1	8	40
1–2	12	45
2–3	30	45
3–4	37	63
4–5	45	64

is a foreshortening of the developmental regions in the root as suggested by the occurrence of the root tip meristem, cell elongation, and the appearance of vascular cells within a region of approximately 0.1 mm.; while in the primary roots of control plants, these developments are found in a region which extends between approximately 0.225 and 5 mm. from the root tip.

Material for a microscopical study of the mature primary root tissue was taken approximately 2 mm. below the region of the root-stem transition. This position below the root-stem transition was accurately determined by making sections back from the hypocotyl. The region thus selected has a well developed pith region and is above the primitive radial root structure. Over 500 comparable sections of control seedlings and of seedlings which had been grown from x-irradiated seeds were examined.

In the transverse sections of control roots (fig 6, top; fig. 9, left) a well-developed secondary vascular cylinder surrounds the tetrarch primary xylem. Lateral roots originate opposite the primary xylem arcs, which at this point, are often occupied by lacunae, bearing scattered peripheral protoxylem elements. The cylinder of xylem is surrounded by a zone of 10 to 12 cells which includes the cambium, phloem, pericycle and endodermis.

In the transverse sections of roots grown from x-irradiated seeds, (fig. 6, bottom; fig. 9, right) the primary xylem not only occurs in its normal tetrarch position, but also forms in a continuous band 2 to 3 cells in thickness between these points and is composed of cells having either the reticulate or scalariform type of wall thickening. No annular or spiral elements appear to be present as occur in the comparable sections of the primary roots of control seedlings.

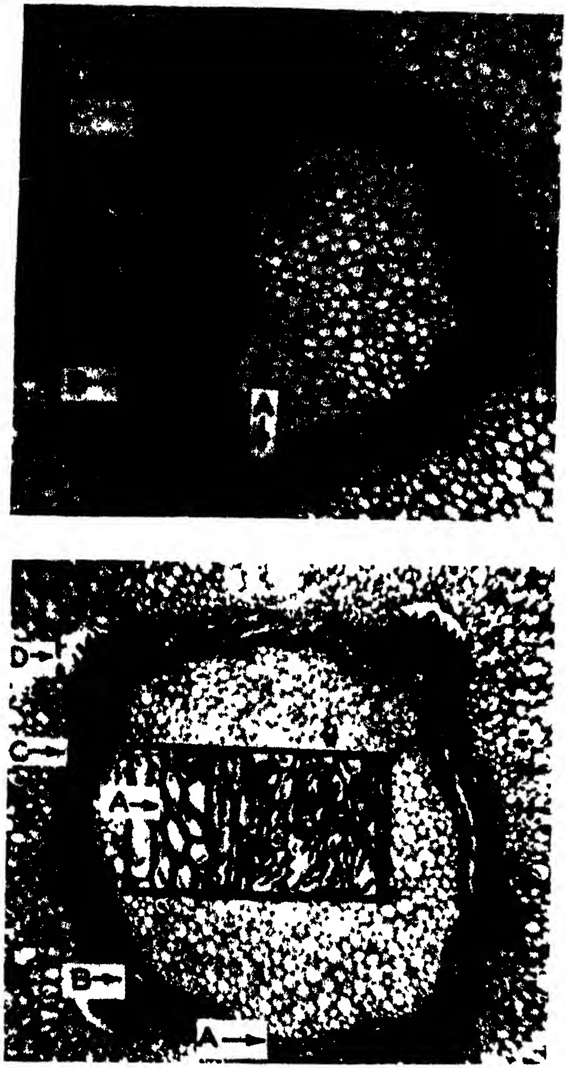

FIG. 6. Mature root development in a control plant of *Vicia faba*, top; and one germinated from an x-irradiated seed, bottom. Primary xylem, A; secondary xylem, B; phloem, C; distorted pericycle, D. × about 150.

The zone of 10 or more cells in thickness (which has previously been described in the control primary root, surrounding the xylem cylinder) is also present, as indicated in transverse section (fig. 6, bottom), in the roots of the plants grown from the irradiated seeds. It is impossible however, to identify the tissue types of this zone because it is undifferentiated and dis-

torted. Its position and small cell size, however, is suggestive of, and corresponds to, a similar region in the primary roots of the control plants which is occupied by the cambium, phloem, pericycle, and endodermis. In the irradiated group, the inner part of this zone is consistently separated from the adjacent continuous primary xylem between the primary xylem arcs, because of a regular distortion of cells in this layer. It lies in contact, however, with a region of larger xylem cells external to the primary xylem arcs which may be secondary xylem. In the primary roots of the control plants, these inner cells (the cambiums) are in close contact with the secondary xylem. The distortion of plant cells which are able to divide (such as those already mentioned of the root tip meristem, and the cambium) is also apparent in a region external to the primary xylem arcs (fig. 6, bottom). This distorted area represents the place where the pericycle had been active in initiating lateral root formation but has now obviously degenerated.

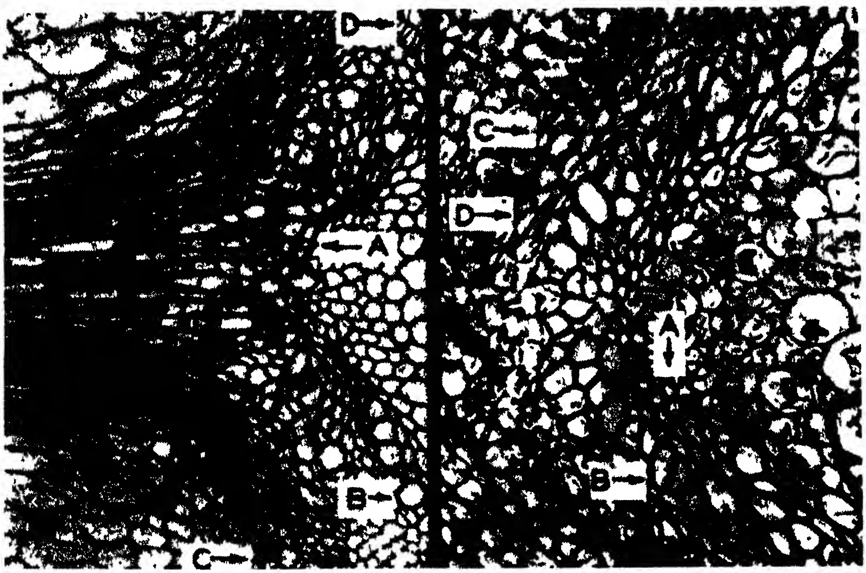

FIG. 7. Comparative developments at the points of lateral root initiation in a normal root of *Vicia faba*, left; and in one germinated from x-irradiated seed, right. Primary xylem, A; secondary xylem, B; phloem, C; distorted xylem, D. × about 900.

One generalization which may be made up to this point, concerning the nature of root modifications which occur in plants of *Vicia faba* grown from x-irradiated seeds, is the partial or complete distortion of tissues whose cellular units are capable of dividing. Evidence of this is seen in the root tip meristem, the cambium, and the pericycle.

Measurement of the distribution of tissues in many transverse sections

of the primary roots of control and of irradiated plants, demonstrates a greater development in the extent of the cortex and of the pith regions in the irradiated group than in the control. This is not caused by an increase in the number of cells as indicated by cell counts, but to an increase in cell size (table II).

TABLE II

A COMPARISON OF ROOT TISSUES IN THE TRANSVERSE SECTIONS OF CONTROL AND IN PLANTS GROWN FROM X-IRRADIATED SEEDS OF *Vicia faba*

REGION	CONTROL	IRRADIATED
	μ	μ
Extent of cortex	900	1098
Extent of pith	1449	2097
Average size of cortex cells	83	99
Average size of pith cells	68	90

The hypocotyl of plants germinated from x-irradiated seeds possesses vascular tissue consisting solely of xylem cells showing the spiral type of wall thickening. These primary elements appear in groups of 2 to 4 cells lying internal to the phloem (fig. 8, right). They are similar in size and

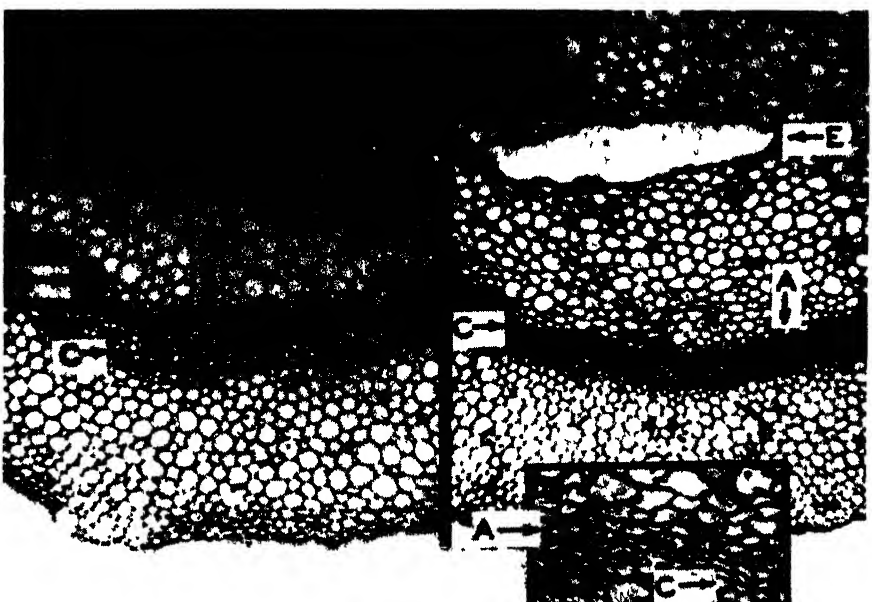

FIG. 8. Transverse sections of the hypocotyl of control seedlings of *Vicia faba*, left; and of plants grown from x-irradiated seeds, right. Primary xylem, A; secondary xylem, B; phloem, C; cambium, D; schizogenous cavity in the pith area, E. × about 150.

position to corresponding primary elements in the control section (fig. 8, left) but not so numerous.

The amount of phloem produced in the rayed hypocotyl is considerably less than that of the control and is poorly differentiated. There is but little noticable variation in the cells of the cortical region of the rayed hypocotyl with the exception of a slight decrease in cell size. Numerous modifications, however, occur in the pith region. There is a marked decrease in both extent of pith and the size of the individual pith cells (table III).

TABLE III

COMPARISON OF PITH SIZE OF CONTROL HYPOCOTYL AND THAT OF A SEEDLING GERMINATED FROM AN X IRRADIATED SEED OF *VICIA FABA*

	CONTROL	IRRADIATED
	μ	μ
Extent of pith region	3240	1520
Size of pith cells	105	51

A most interesting effect of the irradiation observed here is the constant occurrence of a large intercellular cavity in the pith region of the hypocotyl (fig. 10, bottom). This is present when the delayed killing effect is evident

FIG. 9. The origin of the cavity which occurs in the pith area of the hypocotyl of seedlings grown from x irradiated seeds. × about 1500.

in the roots. The cavity first appears in the hypocotyl near the region of the root-stem transition. Figure 9 shows its manner of origin in the pith. The cavity appears to be the result of a schizogenous splitting of the walls of successively formed cells. This abnormal situation may persist for vari-

ous distances in the hypocotyl. In some cases it involves the entire pith area, as shown in figure 10.

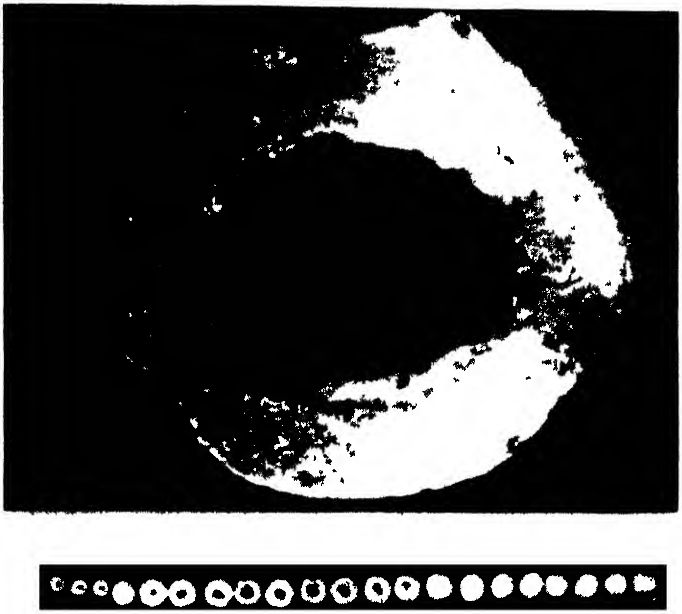

FIG. 10. A large cavity in the hypocotyl of a *Vicia faba* seedling grown from x irradiated seed.

Summary

External and microscopical observations of the primary roots of seedlings grown from seeds of *Vicia faba* (Burpee's bush Lima bean), which had been x-irradiated while unsoaked, are given.

The following external modifications are shown: (a) a general failure of lateral root formation; (b) an apparent poor development of the root tip as compared with controls; (c) the occurrence of "delayed killing" in the roots which becomes evident after a germination period of 8 days.

One thousand slides of the primary roots of germinating x-irradiated seeds and a like number of the primary roots of control plants, were stained and examined using standard methods of microtechnique. A microscopical examination of these slides has given rise to the following anatomical observations:

1. In the germinating irradiated seeds, the root meristem, the beginning of root elongation, and the first occurrence of vascular cells, appear within a span of approximately 0.6 to 0.7 mm. from the root tip; in the control plant, these developments appear over a region of approximately 5 to 6 mm.

2. In the upper limits of the primary root, the primary xylem tissues in the rayed sections appear as a continuous band surrounded by a region of poorly differentiated cells; if compared to tissues at a similar position in the primary root of the control plant, these are comparable to phloem, pericyclar, cambial and endodermal areas.

3. Cambial activity is indicated only at points opposite the primary xylem arcs; here a small band of large xylem cells are found which may be secondary xylem.

4. The degeneration of cells which normally retain their ability to divide is readily observed in the root tip meristem, the probable cambial region, and in the region where lateral roots are normally initiated in the pericycle.

5. Secondary vascular cells are not observed in the hypocotyl.

6. In the hypocotyl, small groups of primary spiral xylem cells appear at intervals internal to a region of cells which may be poorly differentiated phloem.

7. A schizogenous cavity is consistently and characteristically present in the pith region of the hypocotyl of the seedlings grown from the x-irradiated seeds when root growth permanently ceases.

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DEHYDRATION INJURY AND RESISTANCE

G. W. Scarth

(WITH FIVE FIGURES)

Introduction

The causes of dehydration of cells are evaporation, frost, and the osmotic action of hypertonic solutions. In addition to loss of water and consequent diminution of cell volume, there are other effects peculiar to the respective agencies, so that we cannot assume on theoretical grounds that the injury which each of them may cause is effected in the same way. One type of frost injury indeed has no counterpart in the action of drought or hypertonic solutions; namely, disruption of the protoplasm by intracellular ice formation. This, however, occurs only under rather special conditions and at the moment of freezing. It is usually fatal to hardy as well as tender cells. The protection which is developed against intracellular freezing on hardening is an increase of cell permeability which tends to prevent its occurrence (**7**, **8**). This type of freezing is necessarily absent when marked resistance is displayed. Usually ice forms in the intercellular spaces and it is possible that here too it may produce injury otherwise than by dehydration.

Peculiar to hypertonic solutions is their chemical or physico-chemical effect on the plasma membrane. The least toxic, namely sugars and balanced mixtures of Na and Ca salts, are most likely to injure through dehydration. Another difference between osmotic and other modes of dehydration is that generally the protoplast contracts and separates from the wall in the former and wall and all collapse in the latter. Thus the mechanical stresses to which the protoplasm is exposed during contraction and expansion are somewhat different in the two cases.

To throw light on the question of whether frost injury and plasmolysis injury result mainly from the special effects of these agents or from those that they share with drought we may determine the degree of correlation in the resistance of different types of cell to the respective agents.

In the first place it seems to be the rule that extreme resistance to cold, whether in plant or animal, is associated with endurance of desiccation (**6**); perhaps the converse is not invariably true; *e.g.*, myxomycetes (**2**). Quantitative comparison of the degree of dehydration which is lethal with the respective agents and on cells with different degrees of resistance is required to test the relationship more fully. To a certain extent such a comparison is provided by results obtained by the author's collaborators (**5**). All three treatments (desiccation, freezing, and plasmolysis) were applied to sections of similar material and also to the material having different grades

of resistance. In figure 1 freezing points of the solutions in equilibrium with the desiccating atmosphere and of the plasmolyzing solutions are used as a basis of comparison with the actual freezing temperatures in the cold chamber. The sub-zero temperature is a measure of the dehydrating force. The order of resistance of the various tissues, namely, cabbage (petioles), Catalpa and Cornus (cortex of twigs) in the non-hardened state and the same three in the hardened state, is approximately the same with all three treatments. The curves diverge somewhat at the less hardy end of the scale but this divergence could probably be reduced or abolished by regulat-

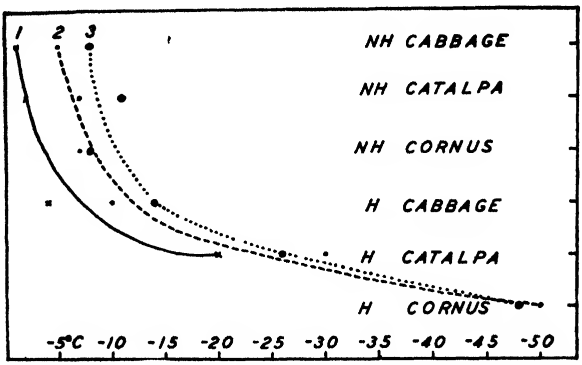

FIG. 1. Freezing points of the solutions in equilibrium with the desiccating atmosphere and of the plasmolyzing solutions compared with the actual freezing temperature in the cold chambers.

Curve 1. Freezing points of the average concentrations of sugar solutions which result in injury on deplasmolysis.

Curve 2. Actual average lethal temperatures in frost injury.

Curve 3. Freezing points of solutions in equilibrium with atmospheres of average lethal relative humidity.

ing the periods of dehydration and equalizing the rate at which water is reabsorbed. Both of these complicating factors are of increasing importance as the cells lack resistance. The time factor operates more rapidly in plasmolysis than frost injury but has not been tested in simple desiccation.

Resemblance between frost and drought resistance is also seen in the fact that both develop together during the hardening process whether induced by low temperature or shortage of water. Moreover, the order of resistance of different tissues is the same with both modes of hardening. An example is shown in the relative desiccation resistance of certain wheat varieties hardened by drought and cold, respectively, as shown in table I from data obtained by WHITESIDE (unpublished results).

Further correspondence between the two types of hardening process is found in the identity of the physiological changes which accompany them. The changes include increase in osmotic pressure, increase in cell perme-

TABLE I

RESISTANCE TO DESICCATION OF LEAF SHEATH CELLS OF SPRING AND WINTER WHEATS

Variety	Drouth hardened plants (5th leaf stage) Molar concentrations of NaCl over which sections were desiccated				
	1.0	1.5	2.0	2.5	3.0
Marquis	++++	+++	0	0	0
Caesium	+++	+++	0	0	0
Quivira*			+++	+	0
Kharkov 22 M C*			++	++	++

Variety	Frost hardened plants (rosette stage) Molar concentrations of NaCl over which sections were desiccated							
	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Marquis	–	–	++	few	+	0	–	–
Thatcher	–	–	++	"	+	0	–	–
Quivira*	–	–	–	–	++++	+++	++	few
Kharkov 22 M C*	–	–	–	–	++++	+++	+++	+

* Winter wheats. Kharkov 22 M C is the hardiest. The spring wheats do not survive at Ottawa.

ability, and a change in the colloidal state and consistency of protoplasm. The latter is of special importance and will be dealt with later.

All these correlations point to a common mechanism of frost and drought injury and a common mode of resistance. Also, inasmuch as resistance to plasmolysis (or sometimes deplasmolysis) injury shows increase with hardening (whether induced by cold or drought) and, as the degrees of dehydration at which sugar solutions and evaporation respectively cause injury are comparable (fig. 1), it would seem that plasmolysis injury is also mainly a dehydration effect.

The principal subject of this paper is the mechanism of injury by dehydration. This problem has been approached by investigating the effect of dehydration on the physical state of protoplasm and the difference between hardy and non-hardy protoplasm in this respect. As it is almost impossible to investigate directly the physical state of the protoplasm in frozen or desiccated tissue, a study was made on plasmolyzed cells. Full details will be published in papers by LEVITT and SIMINOVITCH (5, 9). What follows is a synthetic review of the principal findings and a discussion of their significance.

The methods of study included all of the usual ones for testing protoplasmic consistency; some are applicable to the normally hydrated and some only to the plasmolyzed cells. The criteria are the rate of Brownian movement, rate or degree of displacement of cell parts by centrifuging, rate

of rounding up and shapes assumed during plasmolysis, and the rate of recovery from deformation by micro-needles. In addition two novel micro-manipulation tests proved very instructive. These were based respectively on the flow of protoplasm through punctured pits in the wall (fig. 2), and the shapes assumed by injected oil drops in variously manipulated protoplasts.

Comparison of hardy and non-hardy cells is complicated by the greater, often much greater, osmotic pressure of the hardy. The cells may be compared at the same degree of plasmolysis in which case the liquid phase of

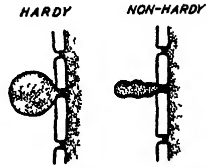

FIG. 2. Comparison of the forms assumed by cytoplasm when expressed through a punctured pit in (a) a hardened and (b) an unhardened cell of Catalpa in a balanced solution of NaCl and $CaCl_2$ (9:1) with an osmotic pressure of 50 atmospheres.

the hardy cells is the more concentrated; or they may be compared in solutions of the same osmotic pressure in which case the hardy cells retain more water. The former compares the effect of equal degrees of dehydration of the cell as a whole, the latter of equal dehydrating forces. High osmotic pressure in itself is a measure of protection against dehydration but does not explain the ability of hardened cells to resist greater dehydration which is their principal safeguard.

Results

The viscosity of the endoplasm or mesoplasm (which is best indicated by Brownian movement and rate of flow) naturally increases as the dehydration proceeds. We usually find little difference between unhardy and hardy cells at equal degrees of hydration (same relative volume) but a decidedly higher viscosity in the unhardy than the hardy, when they are in equilibrium with equal osmotic pressures. This difference results in a greater tendency to injury on deplasmolysis which corresponds in some measure to injury on rapid thawing and on moistening after desiccation.

The ectoplasm[1] or surface layer of the protoplasm shows a more pronounced change on plasmolysis, whether by salt or sugar solutions, than does the mesoplasm. The elastic quality which even normally hydrated and streaming protoplasm reveals on microdissection appears to be mainly a property of the ectoplasm in plant as well as animal cells. With dehydra-

[1] The word *ectoplasm* as used here includes the cortical plasmagel layer as well as the fluid hyaline surface film. The writer now thinks it may be more logical to confine it to the latter—if so ambiguous a term is retained in use.

tion this region becomes increasingly gelatinous and may ultimately lose most of its ductility so that it ruptures when stretched. This is shown by the snapping and crumpling of the ectoplasmic strands which connect a plasmolyzed protoplast with the cell wall (fig. 3) and by the visible rupture

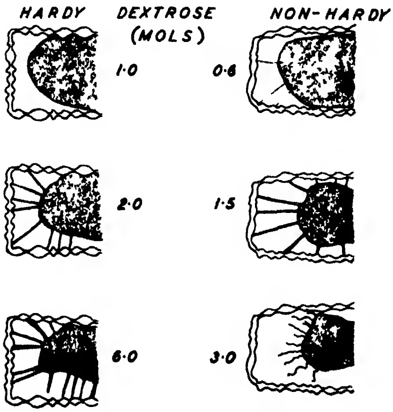

FIG. 3. Relative effect of osmotic dehydration on ectoplasmic strands of hardened and unhardened cells of Hydrangea.

The strands of the unhardened cells snap and crumple in high concentrations (3 M) of dextrose while those of the hardened remain ductile and often flow together in still higher concentrations (6 M).

The hardy strands are invisible in low concentrations and show fainter outline (lower refractive index) than the non-hardy at each stage of dehydration.

of ectoplasm (with outflow of mesoplasm or extrusion of tonoplast) when the cell is stretched by micromanipulation or deplasmolysis. The location of the change is best shown by the behavior of applied oil drops. As shown by CHAMBERS (1), oil drops when brought in contact with a cell surface snap on to the cytoplasm. They assume a lenticular form and appear to lie at the surface but are really covered by an invisible layer of highly surface-active material which spreads over oil as soon as it touches a protoplast. However, the three interfaces—coated oil-medium, oil-cytoplasm and cytoplasm-medium —form definite angles of contact determined by their relative surface tensions. As long as the ectoplasm remains sufficiently fluid the cell can be plasmolyzed and deplasmolyzed or mechanically deformed without permanently upsetting those angles or the shape of the drop. When, however, the ectoplasm sets to a gel its tension increases in proportion as it is stretched so that expansion of the cell surface pulls out the oil drop into a flat and ultimately concavo-convex lens (figs. 4, 5).

Figure 4 compares the behavior of an oil drop in a hardy and a non-hardy cell which have been plasmolyzed to the same degree and then deplasmolyzed. The diagram might represent either a vacuolated cell or a non-

vacuolated such as occurs in the cortex of Cornus twigs. Figure 5 shows that coagulation of ectoplasm occurs in hardy cells of cabbage when the dehydra-

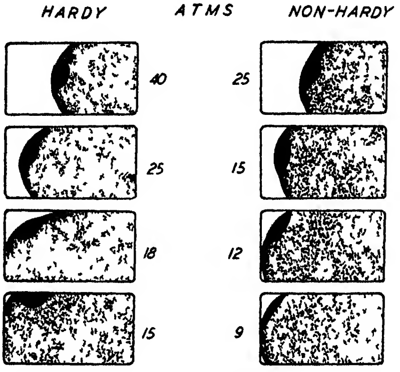

FIG. 4. Effect of deplasmolysis on the form of an oil drop injected into a hardy and a non-hardy cell respectively, *e.g.*, of cabbage (vacuolated) or Cornus (unvacuolated).

In the hardy cell the shape of the drop (black in the diagram) remains unchanged being governed by surface tension forces; in the non hardy it becomes flattened owing to the increasing tension of the solidified ectoplasm when stretched.

tion is carried further but not even saturated invert sugar with an osmotic pressure of over 300 atmospheres coagulates very hardy tree cells.

The relative tendency of their ectoplasms to solidify on dehydration is one of the greatest and probably the most important distinctions between hardy and non-hardy cells. Another striking difference in this connection is that in unhardy cells the solidification increases with the time of exposure while in the most hardy cells the time factor is not apparent at least for a period of many hours.

After a certain stage of dehydration is reached (a stage more or less influenced by the time factor), the colloidal change which underlies the solidification phenomenon becomes irreversible. Then, no matter how gradually water is reabsorbed, the surface layer breaks when its elastic limit of extension is reached. The cells as long as they are undisturbed retain their semi-permeability and appear to be alive but they are unable to regain their normal volume or indeed to survive any mechanical treatment which stretches their surface beyond a certain limit. With hardened cells in which the time factor is negligible up to several hours, a fairly definite critical concentration of solution can be discovered at which the irreversible change occurs in any particular type of cell. As we have seen earlier, there is some evidence that this corresponds to the critical temperature in frost injury and to the critical loss of water in drought injury. If this proves to be generally true a simple and rapid test of frost and drought

resistance will be to determine the concentration of plasmolyte producing incapacity of deplasmolysis.

As noted above the moment of death following plasmolysis does not appear to be that at which the ectoplasm becomes "coagulated" but rather when it ruptures on being stretched during deplasmolysis. In the case of drought or freezing it is possible that not only cell expansion but also further contraction beginning at the point at which coagulation begins, may

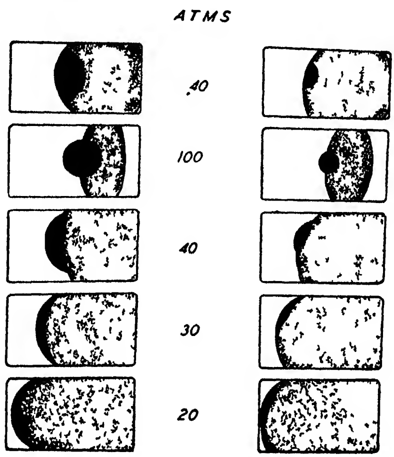

FIG. 5. Solidification of ectoplasm of a *hardy* cell (cabbage) by strong plasmolysis, as shown by changes in a large drop and a small drop during plasmolysis and deplasmolysis. In the lowest figure on the right the layer of cytoplasm is shown underlying the oil drop.

set up damaging mechanical strains in the ectoplasmic layer. Moreover, there is some evidence that severe dehydration may cause the coagulation to become more general. Yet the critical moment is that at which the irreversible change takes place in a thin superficial layer.

What then is the nature of the irreversible change which occurs at the critical stage of dehydration and how does hardening shift the critical point?

Normal ectoplasm is a visco-elastic sol which on dehydration becomes not simply a more viscous sol but rather a more rigid gel. When at a certain stage it changes to an irreversible gel, or coagulum, the inference is that some of its proteins have become denatured and lost much of their hydrophilic quality although the exact physico-chemical mechanism of this transformation is obscure.

As regards the hardening change which might oppose this fatal tendency, there is much evidence of a certain increase in hydrophily of the protoplasm as a whole. Increase in its thickness supports this view (**4**). That the in-

crease of hydrophily is particularly marked at the surface of the protoplast can be inferred from its much lower refractive index in the hardened state. The lower visibility of the ectoplasmic strands represented in figure 3 is one sign of this difference. The increase in permeability of the cell to water and polar solutes is also in harmony with increased hydration of the plasma membrane. The magnitude of the increase in permeability, and also of the decrease in refractive power in very hardy cells, points to greater increase of water content in the surface zone than occurs in the protoplasm as a whole. This result may be due to the greater concentration of colloid in the ectoplasm rather than to a greater change in the character of the colloids there.

Summary

Different kinds and conditions of plant material show parallel variation in their resistance to drought, frost, and plasmolysis, respectively. The maximum plasmolysis that cells can withstand is determined by the point at which an irreversible stiffening, presumably coagulation, of the ectoplasm occurs. The immediate cause of death is usually the rupture of the rigid ectoplasm on deplasmolysis. It is suggested that a similar colloidal change follows upon a certain critical degree of dehydration when produced by frost or evaporation and that with these also mechanical stresses may later cause the fatal rupture.

In hardy cells the ectoplasm has a lower refractive index and higher permeability than in unhardy. These differences point to greater hydration of the ectoplasm the most probable cause of which is greater water binding power of its colloids. The same colloidal difference might account for the greater resistance to coagulation through dehydration.

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EFFECTS OF PERIODS OF WARM WEATHER UPON THE WINTER HARDENED CONDITION OF A PLANT[1]

S. T. DEXTER

Introduction

The influence of alternating periods of high and low temperatures upon the hardening process of plants has been described at various times in the literature (**1, 2, 7**). The rate at which plants lose this hardened condition when exposed to warm temperatures has been investigated (**3, 10, 11**). There appears to be relatively little in the literature, however, concerning the ability of a plant to become hardy a second time, following the dehardening process. Most of the work that relates to this subject is intimately connected with the process of vernalization. The implication from the general theory of vernalization, as stated by LYSENKO, is that, since "every plant passes through a sequence of phases, which replace one another consecutively and in strict rotation," a winter-annual plant, such as winter-wheat, would pass into the hardened condition once, and that, having passed out, it could scarcely reenter that condition. Since this investigation was begun, a number of papers have appeared that deal with this topic indirectly. Among these are KOSTJUČENKO and ZARUBAILO (**8, 9**), who have reviewed literature dealing with the subject of the dependence of winterhardiness on the conditions of seed ripening. If the seeds of rye or wheat were vernalized upon the mother plant during a cold season of ripening, plants grown from them were found lacking in hardiness. Thus, wheat grown in north Russia was found to produce seed that was not suited for planting in that region. since it had been vernalized upon the mother plant, whereas seed produced on plants from the same lot of seed, but grown further south, produced winter hardy plants. GREGORY and PURVIS (**5, 16**) have presented further evidence along the same line. RUDORF (**12**) subjected winter cereals that had been vernalized to varying degrees, to a temperature of 10° C., with various day lengths for various periods of time, and determined the rate of loss of winterhardiness and the degree to which these plants could be rehardened. He concluded that loss of the ability to retain hardiness or to reharden was directly correlated with the degree of vernalization and with the development of the growing point.

Voss (**14, 15**) has presented extensive evidence along the same lines, showing that the degree of vernalization, measured both by microscopic examination of the development of the growing point, and by subsequent growth behavior, is intimately related to the response of the plant to temperatures that ordinarily produce the hardened condition. The length of

[1] Journal Article, new series, no. 440.

day to which the partially vernalized and partially hardened plants were exposed during de-hardening at 10° C. had a great influence upon the development of the growing point, and upon the retention of hardiness. Thus, RUDORF shows the influence of both length of day and of vernalization by low temperatures upon the subsequent development of the plant. DEXTER (**2**) has shown that *unvernalized* wheat plants exposed to a long day, and developing typical upright long-day plants, harden as well as similar plants grown previous to hardening in a short day. Either type of plants hardened better in a long day than in a short day at 2° C. but better in a short than in a long day at 10° C. Sensitivity to the long day, both in development of growing point and in hardening relationships, is shown to be dependent upon previous vernalization. RUDORF's photographs make this very clear. Exposure of vernalized plants to a long day at 10° C. was particularly detrimental to hardening and exposure of unvernalized plants was moderately so.

Experimentation

Alfalfa plants brought into the greenhouse from the field, during the winter, will begin growth immediately as there is no dormant period. In order to avoid the complications arising from such growth, alfalfa roots were severed at the crown. They were cut into pieces about 1 cm. long, washed, rinsed in distilled water, and dried of superficial moisture. A large number of 10-gram samples were weighed into 1″ × 8″ Pyrex test tubes, stoppered, and placed in a water bath at 2° C. One lot was so prepared on October 15, 1936; another similar lot on January 13, 1937; and a third lot on March 15, 1937. Appropriate numbers of tubes with their contained samples were moved to a bath at 12° C., from which they were moved back to the bath at 2° C. as desired. After the desired duration of time at any temperature, 3 tubes with their contents were placed in an alcohol-ice slush at – 7° C. for a 4-hour freezing period. To each tube, 50 ml. of distilled water was then added, and exosmosis allowed to proceed at 2° C. for 20 hours. Electrical conductivity was then determined (**2**). In some cases, field samples were dug and treated in the same manner at intervals. Table I gives the results of these determinations.

As nearly as we are aware, no problem of vernalization enters into the hardening of alfalfa, or into the retention of hardiness by the plant. It has been clearly demonstrated, however, that length of day is a factor in the hardening of this plant (**2, 13**). Since no buds were present on these samples, it was assumed that no growth occurred. The increase and decrease in ability to withstand freezing appear reasonably clear from the table. Without photosynthesis, it seems difficult to reharden the roots, following dehardening, to a condition equivalent to that before dehardening. As has

TABLE I

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 2° C.) OF EXTRACTS OF ALFALFA ROOTS FOLLOWING VARIOUS PERIODS AT THE TEMPERATURES INDICATED. THE ARROWS INDICATE THE PROGRESS OF ANY INDIVIDUAL SAMPLE FROM ONE BATH TO THE NEXT. SAMPLES FROZEN 4 HOURS AT −7° C.; EXOSMOSIS 20 HR. AT 2° C.

Date	2° bath	12° bath	2° bath	Direct from field
Oct. 15, 1936				2304
Oct. 17	2006			
Oct. 20	1378			
Oct. 25		1976		
Oct. 30		2053		
Nov. 4	1438			1635
Nov. 5		1633		
Nov. 6		1911		
Nov. 7		2102		
Nov. 8			1701	
Nov. 14	1788		1543	1262
Nov. 24	1342		1537	1273
Nov. 29			1434	
Nov. 30		1575		
Dec. 1		1795		
Dec. 2		1803		
Dec. 3		2121		
Dec. 4			1797	
Dec. 5			2270—samples deteriorating	
	Second set of samples			
Jan. 13, 1937				1576
Jan. 16		2352		
Jan. 23	1894		2157	
Feb. 2	1997		2059	
Feb. 5		2835		
Feb. 12	1970		2257	
Feb. 22	1822		2284	
	Third set of samples			
Mar. 15, 1938				2173
Mar. 61		2642		
Mar. 20	2408	2762		
Mar. 30			2720	

been demonstrated before (**4**), it is perfectly possible to harden the excised roots materially by simply subjecting them to a temperature of 2° C.

During periods of warm weather, the soil frequently thaws, and some growth may take place. During subsequent cold weather, the ground may again freeze. In the spring of 1937 and 1938, samples were taken in an effort to determine whether dehardening and rehardening occurs in the field.

The tests with samples direct from the field indicate that it is possible for hardening of alfalfa roots to occur following dehardening due to warm weather, but that rehardening is likely to be somewhat incomplete, particularly if any material growth has taken place.

TABLE II

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 2° C.) OF EXTRACTS OF ALFALFA ROOTS DUG FROM THE FIELD UNDER THE WEATHER CONDITIONS DESCRIBED. SAMPLES FROZEN, ETC., AS IN TABLE I

DATE	SPECIFIC CONDITIONS	WEATHER CONDITIONS
Mar. 15, 1937	2160	Frost gone from soil. Crust of frost on surface
Mar. 22	2466	No frost, warmer
Mar. 30	3200	No frost, much warmer, considerable growth
Apr. 6	3057	Snow and colder. 2 or 3″ frost in bare spots
Apr. 8	2532	Still cold and more snow
Apr. 10	2910	Snow gone, warmed up
Apr. 17	3683	Warmer, more growth
Apr. 17	Samples dug, placed in tubes at 2° C. until April 26	
Apr. 26	4453	
Mar. 15, 1938	2172	Soil thawed
Mar. 22	2460	Warmer, some growth
Mar. 30	3195	Still warm, snow and cold that night
Apr. 5	3050	Still snow and cold

WHEAT

In the study of the effects of periods of warm weather on the hardiness and rehardening ability of winter wheat, a different technique was followed. In one experiment, winter wheat plants were grown in the greenhouse at ordinary greenhouse temperatures, transferred to a greenhouse at temperatures around freezing, again placed at the higher temperature, etc., for the periods shown. Samples of crown tissue (leaf sheaths and young leaves) were prepared as usual (**2**), washed, and weighed into test tubes, frozen, and the degree of exosmosis finally determined. In the other experiments, wheat plants were brought in direct from the field where they had been grown under natural conditions in bottomless boxes. These plants, after a period of exposure to greenhouse temperatures, were exposed to hardening temperatures either out-of-doors or in the unheated house, etc. In other cases still, plants were dug from the field for testing before, during, and after periods of warm weather. As in the case of alfalfa, it has seemed desirable to extend this investigation over a period of several years in order to utilize these naturally occurring periods. Table III presents part of the data for experiments involving exposure to warm temperatures in the greenhouse. Since neither size of sample nor freezing temperature were constant from one experiment to the next, the figures given are comparable only, in general, with each individual experiment. Table IV presents the data for a similar study, but with two varieties.

In some cases, there was a growth of several inches during the periods of exposure to warm temperatures. In field-grown samples, the wheat was undoubtedly vernalized. Whenever such growth occurred, rehardening was relatively slight, and in a good many cases, there is no evidence that

TABLE III

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 2° C.) OF EXTRACTS OF WINTER WHEAT CROWNS FOLLOWING PERIODS OF EXPOSURE TO WARM AND COLD WEATHER, AS SHOWN

Date	Specific conductivity: Warm	Specific conductivity: Cold		Remarks
Feb. 8, 1938	2106			Grown in warm greenhouse
Feb. 22		974		After 2 weeks in cold house
Feb. 25	1230			After 3 days dehardening in warm house
Mar. 11		942		After 2 more weeks in cold house—probably vernalized
Mar. 18				Dehardened one week,—much growth in height
Apr. 8		1755		Exposed out of-doors, snow and cold weather, seemingly excellent hardening weather
	From field			
Feb. 25			753	Field grown sample tested
Mar. 3	1292			After one week in warm greenhouse
Mar. 14		1095		After 11 days outside in cold weather
Mar. 17			975	Field grown sample tested
Mar. 24	1810			After one week in warm greenhouse
Apr. 8		1720		After two weeks exposure to cold weather

hardening occurred at all. LAUDE (**10, 11**) has studied the behavior of numerous varieties of winter wheat in coming out of the hardened condition in the spring. He finds that some of the relatively tender varieties, such as Baldrock, above, are relatively slow to come out of the hardened condition, while some of the varieties that can endure low temperatures well (Minhardi) are not well adapted to sharply varying temperatures in the spring. The evidence above adds to his findings. RUDORF (**12**), as well, calls attention to the lack of correlation between hardiness at one stage and hardiness at an-

TABLE IV

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 2° C.) OF EXTRACTS OF WINTER WHEAT CROWNS FOLLOWING PERIODS OF EXPOSURE TO WARM AND COLD WEATHER, AS SHOWN

Date	Baldrock wheat: Cold	Baldrock wheat: Warm	Baldrock wheat: Cold	Baldrock wheat: Field	Minhardi wheat: Cold	Minhardi wheat: Warm	Minhardi wheat: Field
Jan. 11				1980			2070
Jan. 17		2562				2336	
Jan. 21	2576				3050		
Jan. 26		2685					
Feb. 8			2725				
Feb. 18	2560		2605		3030		

other, and suggests: 1. Isolate varieties with a long temperature phase of vernalization and great resistance to cold. 2. Find varieties with unusual hardening ability after temperature phase has been passed through. 3. Find spring grains with great cold resistance.

The plants grown in the greenhouse appeared to harden well on the first two exposures to hardening temperatures. During this four-week period of exposure to low temperature, however, it seems likely that the plants were vernalized. The third exposure to hardening temperature, seems to have been relatively ineffective in inducing hardiness. Table V presents the data taken from field samples following various changes in weather conditions.

TABLE V

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 2° C.) OF EXTRACTS OF WHEAT CROWNS, FOLLOWING EXPOSURE TO VARYING WEATHER CONDITIONS, AS INDICATED

DATE	SPECIFIC CONDUCTIVITY			WEATHER CONDITIONS
Feb. 25, 1938	753			Ground frozen to a depth of a foot or more
Mar. 14	877			Only the crust frozen
Mar. 17	973			No frost, warmer
Mar. 22	1128			Warmer, plants growing; no frost
Mar. 30	1340			Still warm, growth occurring, field green
Apr. 4	1193			Under snow, ground not frozen
Apr. 5	1117			Ground frozen
Apr. 8	1057			Snow left, but ground not frozen
Apr. 10	1200			Snow melted, warmer, crust of frost in A.M.
Apr. 13	1323			Much warmer
Apr. 17	1655			Growing rapidly
	BALDROCK	MINHARDI	RYE	
Mar. 27, 1939	1865	1870	1775	Following warm weather, slight frost in ground. Most leaves previously frozen off, now some green showing
Apr. 1	1060	1488	1310	Cold weather and snow since Mar. 27
Apr. 21	1783	1955	1791	Apr. 1 to 21 mostly cold, with rain and snow. A few days of warm weather. Much greening of wheat, but not much elongation. Maple buds and spring flowers open

From the data taken from field samples, we can conclude that rehardening in the field is possible, even in vernalized plants. When much growth had occurred, however, hardening was decidedly limited.

Discussion and conclusions

In Michigan, winter wheat is rarely injured by severe frosts in the spring after growth has begun. This is not the case, apparently, in Germany (**12**), nor in the Great plains of the United States (**10**), where severe damage can

follow late frosts. In no case in this experiment, even after exposure for a week to greenhouse temperatures, was field-grown winter wheat severely injured by frosts when again exposed out-of-doors. The results of this experiment show that rehardening after vernalization is possible, but indicate that rehardening is more likely in unvernalized plants. When a plant has been vernalized, the tendency toward development and elongation of the stalk is emphasized. As shown in a previous paper, any such tendency is distinctly unfavorable for hardening in the first place (**2**), for retention of the hardened condition (**3**) and, it now appears, is unfavorable for rehardening. The conclusions of this study are, then, in almost complete agreement with the extensive studies of VOSS, RUDORF, and LAUDE. The emphasis that this study may add is that, while rehardening is not rendered impossible by vernalization, it is made less probable. If the development of the wheat plant reaches the stage of elongation of the stalk, the possibility of hardening adequate to withstand ordinary winter temperatures is probably gone.

While vernalization probably does not enter the picture with alfalfa, yet the same general principles apply. Alfalfa appears to reharden following alternate exposure to cold, warm and cold weather, if growth has not occurred to any great extent. Again, however, it is doubtful if, without photosynthesis, full rehardening can be accomplished, since each dehardening appears to permit hardening to a lesser degree. In the mind of the author, these factors can be summed up again (**2**): "The general proposition may be stated that hardening of plants is favored by conditions which tend toward the accumulation or conservation of carbohydrates and other reserve foods; that is, which further photosynthesis and lessen respiration and extension of vegetative parts."

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EFFECT OF MANGANESE DEFICIENCY UPON THE HISTOLOGY OF *LYCOPERSICUM ESCULENTUM*

ETHEL TABER ELTINGE

(WITH THREE FIGURES)

Introduction

The healthy growth of practically all plants requires a small amount of manganese salts. In recent years the symptoms of manganese deficiency have been extensively studied and many new aspects of the question have been revealed. BORTNER (3), HAAS (5), HOFFMAN (6), McHARGUE (10, 11), and others have found that chlorosis may result from manganese deficiency. McHARGUE found less sugar, starch, and vitamin C in plants with insufficient manganese, and GILBERT (4) concluded that there is a relationship between vitamin A and manganese. An important agricultural aspect of the question has been shown by SAMUEL and PIPER (12) who found that the "grey speck" disease of oats is due to deficiency of manganese in the soil. ALBERT (1) found that the addition of manganese sulphate to the soil prevented the appearance of the "grey speck" disease. LEE and McHARGUE (9) found that the application of manganese sulphate to leaves of diseased sugar cane not only cured the disease but increased the growth.

Methods

The purpose of this paper is to present the results of a study of the histology and cytology of plants suffering from manganese deficiency. The plants were grown in nutrient solution made by adding repurified salts to redistilled water and employing 2-liter Pyrex glass beakers as containers. The salts were repurified by the methol of STOUT and ARNON (13). The nutrient solution employed was one commonly used and contained, in addition, traces of boron, copper, zinc, iron, vanadium, nickel, cobalt, titanium, tungsten, chromium, and molybdenum.

Results

The first indication of manganese deficiency appeared on the tenth day as a mottling of the leaves, especially young ones. Within the next two weeks, small necrotic areas appeared in the center of each light green region (fig. 1, B). At first there was no difference in growth rate between manganese-deficient and control plants, but at the end of four weeks, the plants deficient in manganese were noticeably smaller (fig. 1, A). By the end of six weeks both had produced buds, but those on the deficient plants never set any fruit.

These morphological changes caused by manganese deficiency coincided well with those produced internally as observed from sections of fresh leaves and stems put in Ringer's solution and in leaves killed in Nemeč's solution and stained with acid fuchsin and light green. Plastids, especially those in the palisade cells, were the first part of the leaf to show injury. This was first evident as a yellow green color in contrast to the normal dark green

FIG. 1. Tomato plants showing manganese deficiency. A. Six weeks old plants. Left is deficient in manganese. Right, control. B. Mature leaves. Left, control; right, deficient.

of healthy plastids. When treated with iodine, the deficient plastids showed two or three small starch grains, or none at all, while the control plastids were gorged with starch (fig. 2, A, B). At this stage in deficiency there was no difference in thickness of the leaves nor in the size of the cells composing them. When the leaves began to show necrotic areas externally, these were evident internally (fig. 2, C, D). At first isolated palisade cells here and there among yellow green cells were the only necrotic cells, then groups of palisade cells, and finally the injury spread to the epidermis and spongy parenchyma until the whole cross section was involved (fig. 3, A). At this advanced stage in deficiency, control leaves not only had longer and, in general, wider palisade cells filled with healthy green plastids, but were also much thicker than deficient leaves (fig. 2, C, D). In the latter there were a few normal plastids in the palisade cells near necrotic ones, but most of the plastids that were left had begun to show vacuoles within them (fig. 3, D, E), or had become yellow green in color and indistinct as to outline (fig. 2, D). When the brown area included most of the cross section, or at least a group of cells, there were seldom any cells near by with healthy

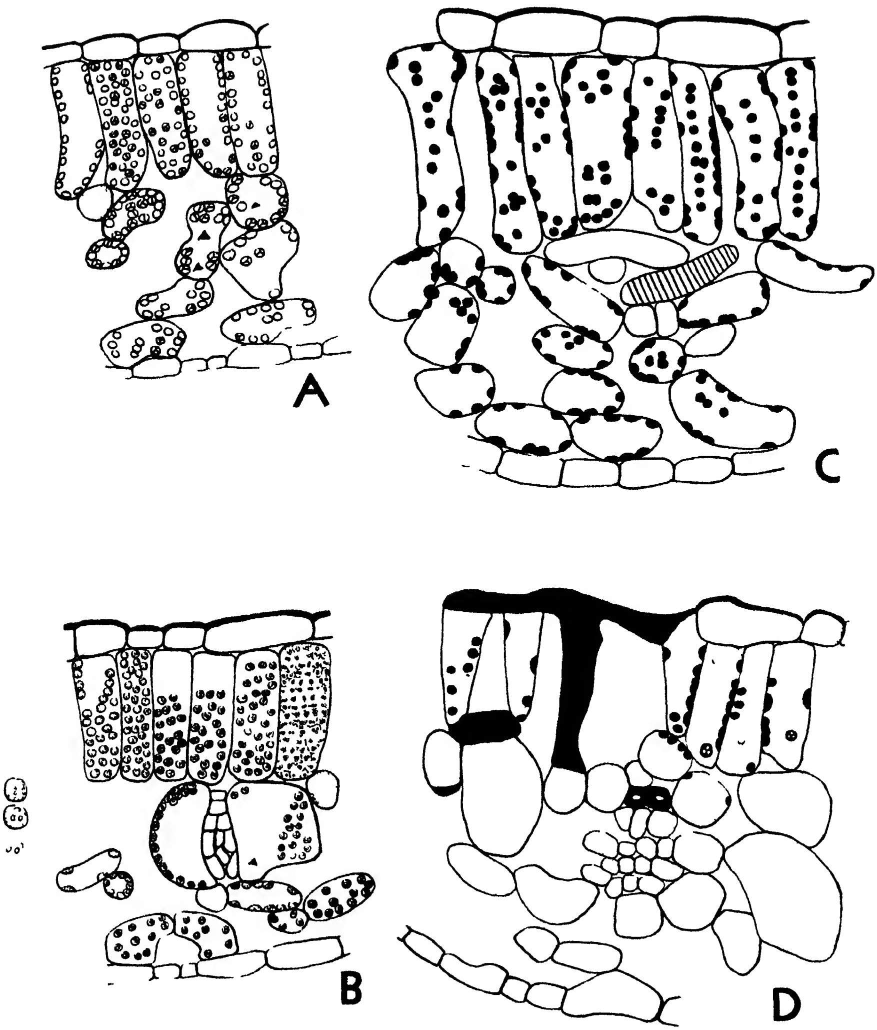

FIG. 2. Cross sections of tomato leaves. A. Control leaf × 240 showing large starch grains within the plastids. B. Manganese deficient leaf × 240 showing some plastids with no starch grains, some with 2–3 small ones and some plastids with very indistinct outline but with 2 or 3 small starch grains. On the left is an enlarged plastid of each of the three types mentioned. C. Healthy leaf × 339. D. Deficient leaf showing necrotic cells colored black. Some plastids show vacuoles, others are very indistinct in outline, × 339.

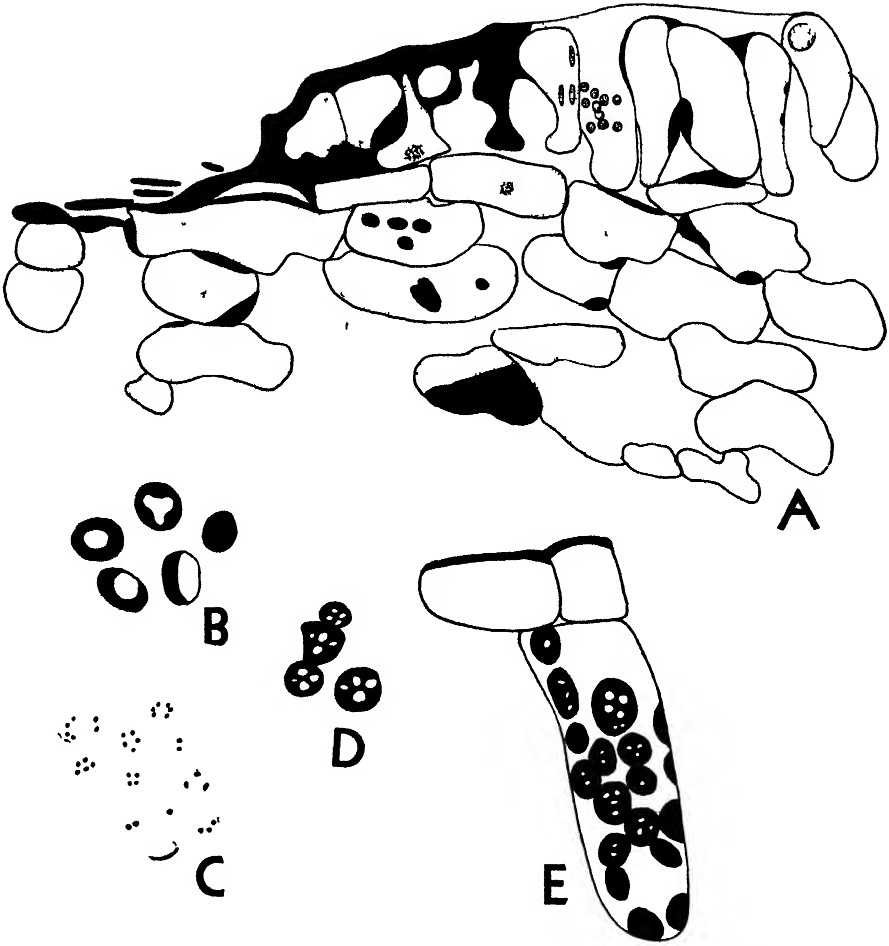

FIG. 3. A. Cross section of leaf, ×276, showing advanced deficiency. Most of the plastids have disintegrated and many cells are necrotic. B. Normal plastids treated with silver nitrate, ×630. C. Deficient plastids treated with silver nitrate, ×630. D. Plastids showing large vacuoles from near a necrotic region, ×604. E. Palisade cell showing vacuolization of plastids caused by manganese deficiency.

plastids. Occasionally there was a cell with a few yellow green granular ones. Often the cells that were not necrotic showed only coagulated material staining red with acid fuchsin (fig. 3, A).

A deficiency in manganese in addition to producing structural changes, brought about the formation of abnormal products of metabolism. Healthy tomato leaves showed various types of crystals. These frequently filled a cell, especially in the spongy parenchyma. Manganese-deficient leaves on the other hand often showed regions where every palisade cell was at least

half filled with small calcium oxalate crystals. When deficient leaf sections were placed in indol phenol blue, oil was found in all cells in badly affected areas, while in control leaves it was found only in the epidermis and hairs. Dilute soap solution applied to cross sections of fresh deficient leaves caused the granular yellow green plastids to dissolve leaving a clear yellow green solution within the cell. If the granular plastids had small starch grains within them, these could be seen moving about within the green solution. Sections of control and deficient tomato leaves were placed in a two per cent. solution of silver nitrate according to the method of WEIER (**14**). These sections were cut within two hours after bringing the plant into the laboratory from the bright sunlight of the greenhouse. Plastids from healthy leaves showed wide bands of reduced silver nitrate partly or all the way around the edge of the plastid (fig. 3, B). Occasionally a plastid was entirely brown-black. Macroscopically, the entire section looked black. Plastids from the deficient leaves were indistinct in outline. Close observation showed very small dark brown granules within some of them. These granules were irregular as to number and position (fig. 3, C). Macroscopically, these sections looked slightly darker than untreated ones but distinctly not black.

Tomato plants exhibiting advanced deficiency often showed some of the younger leaves and leaflets wilting. In section such a leaflet usually showed some dead cells and a few cells with some plastids, many of which were granular or vacuolated. Sections through small veins showed some of the cells partially filled with crystals and a few cells completely full of granular material staining pink with acid fuchsin.

The stems of manganese-deficient plants showed, in general, the same differences as did the leaves. They were smaller in diameter and contained a much narrower band of xylem tissue. Fresh sections stained with methyl green indicated that the newest xylem was still alive. Much of the older xylem, however, was dead. The older xylem had many cells which were torn in outline. This was not true of similar control sections. In deficient stems, as well as leaves, more parenchyma cells were filled with crystals. Stems from plants showing leaflets wilting, often showed some of the xylem cells plugged with brown material. A few of the xylem cells were partially filled with crystals. Manganese-deficient stems had some starch stored within them but the grains were much smaller and usually simple in contrast to the large compound ones found in control stems.

Discussion

From the results of this work it is evident that a lack of the element manganese brings about some of the same changes histologically that does a toxic amount of the element. KELLEY (**7, 8**) in his work with a toxic quan-

tity of the element reported a chlorosis in pineapples followed by first a decrease in number and size of plastids and finally by their dissolution. Along with this went a decrease in the amount of starch and sugar and the formation of excessive numbers of calcium oxalate crystals.

In the present investigation the chloroplasts also appeared to be the first part of the plant injured. Something happened within the plastid to stop the manufacture of chlorophyll. This resulted almost at once in the decrease in size and number of starch grains. The plant was not able to use this starch completely but changed part of it to fat which was present in all cells of the leaf. Along with the change from starch to fat went first the vacuolation and then dissolution of the plastids. With the disruption of the chlorophyll mechanism, the ascorbic acid content of the plants was reduced almost to zero. Here, as with the toxic amount of the element, calcium oxalate was found in large amounts; many cells were filled with crystals of all sizes.

Summary

1. Chloroplasts are the first part of the plant affected by a deficiency of manganese. They become yellow green in color, gradually lose their starch grains, become vacuolated, then granular, and finally distintegrate into an indistinct mass along with the cytoplasm. This eventually turns brown. Plastids deficient in manganese dissolve in dilute soap solution. They also produce very little, if any, ascorbic acid.

2. Manganese-deficient leaves are thinner and have smaller palisade cells than do control ones. Many more cells in the former contain large masses of crystals than do those in the latter. This is also true of the parenchyma cells in deficient stems.

3. Manganese-deficient stems are smaller in diameter, contain less xylem, and often show xylem cells plugged with coagulated material. Some of the conducting cells in the veins of deficient leaves are also plugged. Here the clogging is caused by both crystals and coagulated material.

4. Some of the food stored in deficient leaves is in the form of fat. That in stems is in the form of starch but the grains are mostly simple and few in number compared to those in healthy stems which are compound and numerous.

The writer wishes to express sincere appreciation to PROFESSOR HOWARD S. REED for advice and help during the preparation of this paper, and also to PROFESSOR DENNIS R. HOAGLAND, Division of Plant Nutrition of the University of California, under whose direction the plants were grown.

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TOXICITY OF SELENIUM-CONTAINING PLANTS AS A MEANS OF CONTROL FOR RED SPIDERS[1]

V. H. MORRIS, C. R. NEISWANDER, AND J. D. SAYRE

Introduction

One of the difficulties frequently encountered by investigators in growing plants in greenhouses is the control of the common red spider, *Tetranychus telarius* L. Corn plants are especially suspectible, and in a series of physiological studies at Wooster, Ohio, red spiders threatened to become a limiting factor. Insecticides suitable for use on corn plants were applied with knapsack, wheelbarrow, and high-pressure orchard sprayers, but did not give satisfactory control.

The observations by HURD-KARRER and POOS (4) that red spiders were unable to persist on cereal plants growing in nutrient and soil cultures containing available selenium seemed to offer a new approach to the problem of red spider control. Although selenium is extremely toxic to both plants and animals in high concentrations, most plants are known to tolerate low concentrations. The problem then was to find concentrations of the selenium salt which would not be toxic to the plants and would allow the accumulation in plant tissues of levels of selenium sufficient to be toxic to red spiders.[2]

Methods

In the studies reported in this paper, corn plants were grown by "tank culture" technique in cement tanks 10 feet long, 2½ feet wide, and 8 inches deep with a capacity of 470 liters of nutrient solution. Twenty-four plants were grown in each tank, supported on hardware cloth trays filled with excelsior. Oxygen was supplied to the roots by frequent aeration of the nutrient solution, and the reaction was kept between pH 5.5 and 6 by occasional adjustment.

The composition of the nutrient solutions used in the several experiments differed slightly, mainly in the relative proportions of $Ca(NO_3)_2$ and KNO_3. Essentially, however, the composition, expressed as millimols per liter of nutrient solution, was as follows: KH_2PO_4, 0.53; $Ca(NO_3)_2$, 10.86; KNO_3, 6.98; $MgSO_4$, 2.74; and $(NH_4)_2SO_4$, 1.43. Frequent additions of small

[1] Based on investigation cooperative between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Departments of Agronomy and Entomology, Ohio Agricultural Experiment Station.

[2] Since this manuscript was prepared for publication, LEUKEL (5) reported the use of selenized soil in the control of aphids and red spiders on sorghum.

quantities of $MnSO_4$, H_3BO_3, and $FeSO_4$ were made. The solutions were completely renewed at two- or three-week intervals.

The form of selenium used in these studies was sodium selenate. In the preliminary experiments reagent quality $Na_2SeO_4 \cdot 10\,H_2O$ was employed; a much cheaper source was discovered in a technical grade of anyhdrous sodium selenate, however, and used in the subsequent experiments.

At the completion of each experiment the plants were harvested, cut up in an electric food chopper, and aliquots dried for analysis. The procedures used for the determination of selenium were those devised and employed by the Food and Drug Administration, United States Department of Agriculture (**1**, **6**).

Results

Preliminary experiments

In the first of two preliminary experiments red spider populations were reduced by two additions of sodium selenate. The first addition supplied 2, and the second 3, p.p.m. of selenium to the nutrient solution.

In the second preliminary experiment, 2 p.p.m. of selenium were added to the nutrient solution at 10-day intervals. When the mature plants were harvested, the plants in the control tank were practically dead as a result of spider injury, whereas the plants receiving selenium were nearly free of red spiders.

There was no indication of toxicity to the plants in either test and the results were, therefore, encouraging. To test further the possibility of controlling red spiders by selenium-induced toxicity without disturbing normal plant development, a more elaborate series of experiments was undertaken and is reported in detail.

Experiment I

On October 25, 1937, a series of four tanks was planted to a single-cross hybrid. One tank was used as a control and received no selenium. Selenium was added to the other three tanks at the rate of 0.5, 1.0, and 2.0 p.p.m.[3] Beginning two weeks after planting, five additions of selenium were made at approximately two-week intervals. In order to obtain a uniform distribution of red spiders, the plants were artificially infested December 13 and were harvested January 28. Twelve plants were composited for each sample for analysis. Observations made on the red spider infestations and on the condition of plants at time of harvest, together with the selenium content of the samples, are given in table I.

[3] The sodium selenate used in these experiments was of technical grade, purchased from the Harshaw Chemical Co., Cleveland, Ohio. At the time of these first experiments we were not equipped to determine its purity, and it was accepted as 100 per cent. Later analysis showed only 85 per cent. selenate, so that these treatments were actually 15 per cent. less than the figures given.

TABLE I

EFFECT OF DIFFERENT CONCENTRATIONS OF SELENIUM IN THE CULTURE SOLUTIONS UPON THE SELENIUM CONTENT OF WHOLE CORN PLANTS AND ON RED SPIDER INFESTATION. DATA CALCULATED ON A DRY MATTER BASIS

TREATMENT	SELENIUM CONTENT	OBSERVATIONS ON RED SPIDER INFESTATION AND CONDITION OF PLANTS
	p.p.m.	
Control	2	Thoroughly infested, practically dead from red spider injury.
Selenium 0.5 p.p.m.	31	Well infested, severe injury on lower leaves, some injury to upper leaves.
Selenium 1.0 p.p.m.	56	Numerous dead red spiders, no red spider injury.
Selenium 2.0 p.p.m.	87	No red spiders, no red spider injury.

EXPERIMENT II

The tanks used in this experiment were planted to a single-cross hybrid February 12.

The selenium additions, which were at the same rates as in the preceding experiment, were begun one week after planting and continued weekly throughout the growth period. To ensure uniform red spider infestation the plants were artificially infested May 9. The plants were harvested June 30. Composite samples of the leaves from 12 plants from each tank were taken for analysis. Observations on the red spider infestation and on the condition of the plants at harvest, together with their selenium content, are presented in table II.

TABLE II

EFFECT OF DIFFERENT CONCENTRATIONS OF SELENIUM IN THE CULTURE SOLUTIONS UPON THE SELENIUM CONTENT OF CORN LEAVES AND ON RED SPIDER INFESTATIONS. DATA CALCULATED ON A DRY MATTER BASIS

TREATMENT	SELENIUM CONTENT	OBSERVATIONS ON RED SPIDER INFESTATIONS AND CONDITIONS OF PLANTS
	p.p.m.	
Control	5	Leaves thoroughly infested, plants dead.
Selenium 0.5 p.p.m.	23	General infestation, red spider injury rather light.
Selenium 1.0 p.p.m.	46	Few living red spiders, survivors abnormally sluggish in movement, no red spider injury.
Selenium 2.0 p.p.m.	101	No living red spiders, no red spider injury.

EXPERIMENT III

A third experiment was carried out during the summer of 1938. The previous experiments had indicated that the toxicity of corn leaves grown in a nutrient solution receiving 0.5 p.p.m. of selenium was inadequate for

protection from infestations of red spider. The selenium levels for this experiment were accordingly increased to 1.0, 2.0, and 3.0 p.p.m.

The corn was planted July 6. Each tank contained a set of five commercial hybrids replicated four times. The selenium additions were begun immediately and continued weekly throughout the growth period. The plants were artificially infested September 3. When the plants were harvested October 5, the leaves and stems of five plants in each replication were composited separately for analysis.

The selenium content of the samples and the observations made at harvest on the effect of treatments are presented in table III.

TABLE III

EFFECT OF DIFFERENT CONCENTRATIONS OF SELENIUM IN THE CULTURE SOLUTIONS UPON THE SELENIUM CONTENT OF CORN TISSUES AND ON RED SPIDER INFESTATION. DATA CALCULATED ON A DRY MATTER BASIS

Treatment	Selenium content		Observations on red spider infestations and conditions of plants
	Leaves	Stems	
	p.p.m.	*p.p.m.*	
Control	2	1	Leaves thoroughly infested, red spider injury causing death.
1 p.p.m. selenium	51	19	Few living red spiders, no red spider injury.
2 p.p.m. selenium	133	49	No living red spiders, no red spider injury.
3 p.p.m. selenium	196	56	No living red spiders, no red spider injury.

The plants in the control tank were so thoroughly infested with red spiders that the lower surface of the leaves were completely covered with webs. The tank receiving the 1 p.p.m. treatment was separated from the control tank by a space of only 18 inches and the leaves of the plants from the two treatments intermingled. As a result there was considerable migration from the heavily infested and dying control plants to those receiving 1 p.p.m. of selenium. Although the presence of many red spiders, including young and eggs, could be observed on the leaves of the plants receiving 1 p.p.m. of selenium, close examination showed that except for an occasional sluggish individual, they were dead.

During the summer a relatively heavy infestation of aphids, *Aphis maidis* FITCH, developed in the tassels of the control plants; none was observed on plants receiving selenium.

ADDITIONAL EXPERIMENTS

The successful control of red spider in these experiments with corn led to the use of selenium in various other plant nutrition studies at the Ohio

Agricultural Experiment Station. These studies included many diversified investigations, such as mineral deficiency studies with young apple and elm trees, tests of commercial legume inoculation cultures, and the growing of wheat plants for hybridization purposes. Sand and soil cultures were employed in these studies and the rate of selenium addition varied from 1 to 3 p.p.m. in sand culture to 10 p.p.m. in soil culture. Previously in these studies it had been necessary to combat red spider constantly, and control was at best uncertain. The use of selenium, however, eliminated the red spider problem in each of these investigations.

Discussion

A factor which must be considered in the use of selenium is the selenium-sulphur antagonism reported by HURD-KARRER (**2**, **3**). She found that the toxicity of selenium to plants and the quantities of selenium absorbed varied with the proportionate concentrations of available sulphur and selenium. According to HURD-KARRER, toxicity to plants is not likely to occur when the quantity of selenium is less than the ratio 1 of selenium to 12 of sulphur. The nutrient solutions used in these studies with corn varied in sulphur content between 130 and 190 p.p.m, depending upon whether tap water or rain water was used in making up the solutions. Thus the selenium-sulphur ratios in the nutrient solution varied from approximately 1:200 to 1:40. In no case was there observed the slightest evidence of toxicity to the corn plants.

Under the conditions of these experiments with additions of 0.5, 1.0, 2.0, and 3.0 p.p m. of selenium to the nutrient solution in which corn was grown, leaf tissues contained approximately 25, 50, 100, and 150 p.p.m. of selenium, respectively. The leaf tissues containing approximately 25 p.p.m. of selenium were sublethal to red spiders although the infestations did not cause much injury; at 50 p.p.m. red spider infestations were inhibited although occasional sluggish individuals were found. At concentrations approximately 100 p.p.m. or above, red spiders were unable either to multiply or persist. The experiment in which a natural infestation of aphids occurred on plants which did not receive selenium but did not occur on plants receiving selenium indicates that aphids are unable to persist on plants which have an available supply of selenium.

It should be emphasized that selenium is recognized as one of the very toxic poisons to man and animals and the use of selenized tissue as a means of insect control should be confined to plants intended for experimental or non-food purposes.

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LACK OF INHIBITION OF LATERAL BUDS BY THE GROWTH-PROMOTING SUBSTANCE PHENYLACETIC ACID

FELIX G. GUSTAFSON

(WITH TWO FIGURES)

It is now a well-known fact that indole acetic and indole butyric acids, when applied in lanolin to cut apices of stems suppress the formation of lateral buds, as do the terminal buds themselves. THIMANN and SKOOG (9) were the first to demonstrate this when they applied an extract from *Rhizopus suinus* to decapitated *Vicia faba* seedlings and found that the lateral buds were inhibited. It was later found that the active substance was indole acetic acid. Since that time other investigators have demonstrated the same thing for other plants (**2, 3, 6, 7, 8, 9, 10**).

In some laboratory experiments with *Helianthus annuus* the writer applied indole acetic acid and phenylacetic acids in lanolin to the cut surfaces of stems. After some time it was noticed that some of the treated plants had produced lateral buds while others had not and when a check was made it was found that the treated plants that produced shoots were the ones that had been treated with phenylacetic acid.

This interesting observation led to a more detailed and carefully controlled experiment. *Helianthus annuus* was used again because under normal treatment it never produces any lateral buds or branches. Four lots of 16 plants each were carefully selected, besides some control plants not decapitated. These plants were between 60 and 70 centimeters tall; no flower buds had as yet been formed. Ten to 15 cm. of the top were cut off from each one of these 64 plants. The cut was always about one centimeter above a node. One per cent. indole acetic, indole butyric, and phenylacetic acids mixed separately in lanolin, as well as lanolin alone, were used. Sixteen plants were treated with each chemical on March 20, 1939. A considerable amount of material was smeared on the freshly cut surface, but it was not repeated during the time of the experiment.

On April 10 representative plants from each group were photographed (fig. 1). At that time the plants treated with the auxin showed the typical gall formation, but no buds or lateral shoots had been formed on any of the plants treated with indole acetic and butyric acids. The plants treated with phenylacetic acid had on the other hand formed as many and nearly as large buds as the plants treated with plain lanolin. A month later (May 13) representative plants from each lot were again photographed (fig. 2). As the photograph shows, all of the plants except those not decapitated had by now produced some buds or branches. It is to be remembered that during a period of 54 days no new auxin application had been made. By

now the lateral branches of the lanolin and phenylacetic acid treated plants had produced flower buds. Most of the plants to which indole acetic acid had been applied had several branches, that were much larger and more numerous than those on the plants treated with indole butyric acid. It thus appears that phenylacetic acid does not suppress lateral bud growth at all, while indole acetic and indole butyric acids do, and the latter seems to be more effective than the former.

Not until a thorough search was made of the literature was it realized that phenylacetic acid had been used in a similar experiment before. In a paper published in 1935 Hitchcock makes the statement: "If the cut surface of decapitated stems (tobacco) was treated with a lanolin preparation

Fig. 1. Helianthus plants 21 days after the commencement of the experiment. Reading from left to right, the plants were treated with pure lanolin, indole butyric, phenylacetic, and indole acetic acids. Only plants treated with lanolin and phenylacetic acid have buds.

containing a high concentration of any one of the seven growth substances (indoleacetic, indolebutyric, indolepropionic, phenylacetic, phenylpropionic, phenylacrylic, and naphthalene acetic acids) used in the bending tests, marked inhibition of the growth of the upper two or three buds occurred. . . . No attempt was made to compare the effectiveness of the different substances in these tests." Thus, according to Hitchcock, phenylacetic acid inhibits the bud development in the tobacco plant. The writer has not repeated this experiment with tobacco. Recently, one of the writer's students has repeated the above experiment on *Helianthus annuus* with several hundred plants for each chemical and in every instance has he found that phenylacetic acid does not inhibit bud formation. It produces a gall like the other sub-

stances, but no bud inhibition. Thus there is no question about its lack of inhibition of lateral buds in Helianthus.

If we compare other growth-promoting activities of phenylacetic acid with other growth substances we find the following. According to WENT and THIMANN (**11**), phenylacetic acid is about ten per cent. as active in the pea test as is indole acetic acid, and only 0.02 per cent. as active in the Avena test as indole acetic acid, which means that for practical purposes it does not cause Avena curvatures. ZIMMERMAN and WILCOXON (**12**) found that phenylacetic acid, though less effective than indole acetic and indole butyric

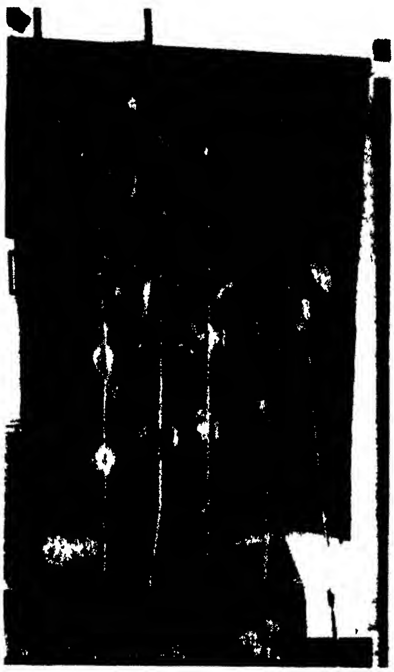

FIG. 2. Plants 54 days after the beginning of the experiment. Reading from left to right, the plants were treated with lanolin, indole butyric, phenylacetic, indole acetic acids, and no treatment. Now all of the treated plants have buds, but the ones treated with indole butyric acid have much smaller buds.

acids, does cause root production, negative bending of stems, epinasty of leaves, and suppresses stem elongation. AVERY and SARGENT (**10**) found that phenylacetic acid does not increase the straight growth of Avena coleoptiles, but actually inhibits it. GUSTAFSON (**4, 5**) has shown that phenylacetic acid is about as effective as indole acetic and indole butyric acids in inducing parthenocarpy and more effective than indole acetic acid in causing size growth of tomato fruits.

We thus see that in most of its effects on plant growth phenylacetic acid is less effective than indole acetic or indole butyric acids. This is, however, not true as far as parthenocarpy is concerned.

This note has been written because it seems to the writer that the interesting behavior of phenylacetic acid with respect to gall formation but lack of lateral bud inhibition might be of considerable value in the experimental study of bud inhibition.

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BRIEF PAPERS

AUTOMATIC WATERING OF EXPERIMENTAL PLOTS

KENNETH POST

(WITH TWO FIGURES)

Various methods of maintaining uniform moisture in the soil for young plants are used by experimentalists. Probably weighing the container in which the plant is growing, from time to time, and adding sufficient water to bring it to a definite weight meets with favor in the majority of cases.

JOHNSTON and ATKINS (3) developed a system for automatic control of adding water periodically to plants in pots. CALFEE and MCHARGUE (2) developed a flower pot which utilized the principle of capillarity in supplying water.

Greenhouse experiments conducted in large beds or benches with volumes of soil of sufficient size to make watering by these methods impractical are more difficult to keep at constant or equal moisture. Soil tensiometers described by RICHARDS, RUSSELL, and NEAL (6) or conductivity methods suggested by BOUYOUCOS and MICK (1) and WHITE-STEVENS and JACOB (7) serve to determine the soil moisture content. The water is then added when a given moisture content is reached. Great fluctuations in water content of the soil thus occur and it is difficult to uniformly apply the water over the area in question.

Some of this difficulty is overcome in the automatic control here described. A water-tight, level bench (fig. 1) is provided with two rows of

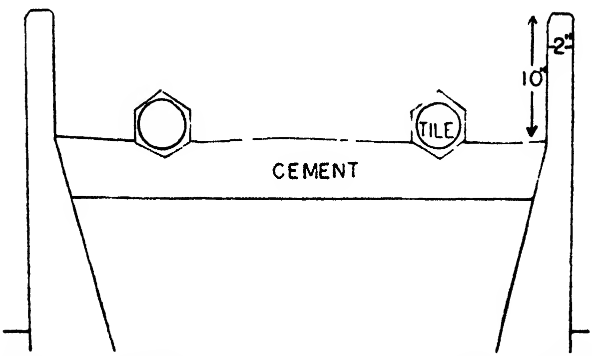

FIG. 1. Cross section diagram of bench construction for sub irrigation of bench plants.

tile running lengthwise. The tile slope one inch in 100 ft. and are connected by T's in the middle of the bench. This construction has previously been described by the writer (4).

A soil tensiometer is connnected to a Bourdon vacuum gauge (fig. 2). A piece of copper wire is bent and fastened to the inside of the celluloid face of the gauge. It is placed so that the hand will touch it on its upward movement as the soil tension increases. The ground wire is fastened to the base of the gauge.

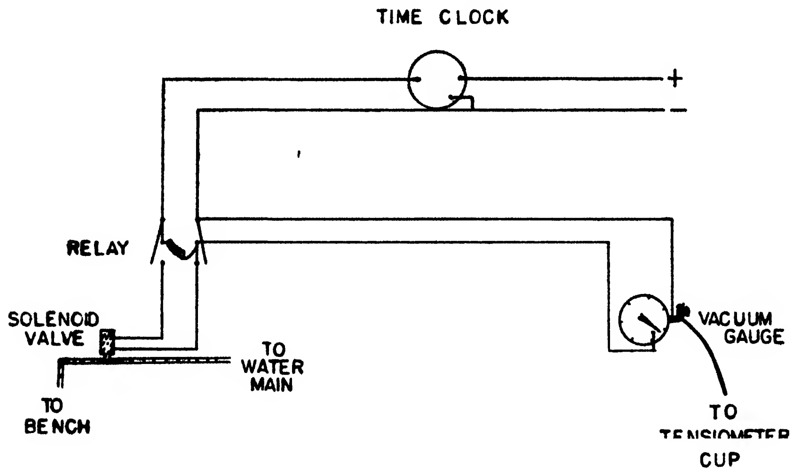

FIG. 2. Wiring diagram for automatic watering of experimental plots.

The tensiometer operates a relay which in turn operates a solenoid valve and injects water into the tile in the bench. The electrical current flows through a time clock which permits it to flow to the vacuum gauge at two hour intervals. The period of flow of the current is determined by the time required to inject the desired amount of water in the bench.

The two-hour time interval was calculated after several trials to determine the time required for the absorption of the water, and its movement to affect the tensiometer.

The distribution of water in the soil has been found to be uniform through the bench when a low tension (5 to 15 cm. of mercury) is maintained. Capillarity is not as uniform at high tensions and the soil moisture is not as uniform. The lower layers are moister than the surface.

The automatic watering method is satisfactory when one it attempting to grow benches of plants for fertilizer, light, temperature, and other studies necessitating equal moisture in the plots.

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COMPARATIVE TRANSMISSION SPECTROGRAMS OF AN IRRADIATED LEAF EXTRACT

FREDERICK F. FERGUSON AND LEWIS W. WEBB, JR.

(WITH ONE FIGURE)

This paper is a preliminary effort to show something of the spectrographic changes which occur upon radiation of an alcoholic solution of leaf green. WEBB and FERGUSON have previously indicated the nature of the changes caused by dilution, in the spectrograms of an alcoholic leaf extract.

The material used in this study was prepared in the following manner: 3 grams of damp grass (*Poa pratensis*) were refluxed with 100 ml. of methyl alcohol for 20 minutes. Ten ml. of the resulting dark green mixture were then diluted with 90 ml. of methyl alcohol.

The transmission spectrogram of a sample of this solution was then obtained.[1] This solution was next removed from the spectrophotometer and subjected to the radiation from a 200-watt unfrosted bulb. The center of the specimen tube was *ca.* 15 cm. from the outside of the lamp. The radiation was continued for two hours and the solution was again tested. About one hour was required for each set of observations obtained by using the spectrophotometer, during which time some radiation was naturally furnished by the instrument. The other graphs were taken by continuing this procedure. It will be noted here that curve no. 1 is that of a dilute solution of leaf green possessing the characteristic spectrogram as shown by FERGUSON, DELOACH and WEBB (1) for an ethyl alcohol solution.

As the radiation was continued upon the sample many pronounced changes occurred. One noticeable feature was the gradual visual transformation from a green to a brownish flocculent fluid. This color change can be easily seen by the proper interpretation of the graphs in figure 1.

An examination of these graphs also shows an increase in the ability to transmit light in all of the wave bands except from *ca.* 500 mμ to *ca.* 580 mμ and in the infra red. Transmission is gradually increased at *ca.* 360 mμ. There is a marked tendency for all five curves to meet at *ca.* 480 mμ, 580 mμ, and 690 mμ. The extremely high transmission in the infra red is a factor common to all of the curves. The decrease of absorption for wave-bands in the region of *ca.* 660 mμ is comparable to that caused by increased dilution of an alcoholic leaf extract as shown by WEBB and FERGUSON (2). The relative high loss of transmission from *ca.* 500 mμ to *ca.* 580 mμ and from *ca.* 700 mμ to *ca.* 900 mμ is of interest. Curves nos. 3 and 4 coincide in the region of *ca.* 530 mμ to 580 mμ and 740 mμ to 900 mμ. With the exception

[1] The instrument used in this study was the Coleman Regional Spectrophotometer.

of the region of *ca.* 360 mμ to 480 mμ curves nos. 4 and 5 are in entire agreement.

The spectral range for the curves extends from 360 mμ to 900 mμ. Each point represents the average transmission of a band 30 mμ wide.

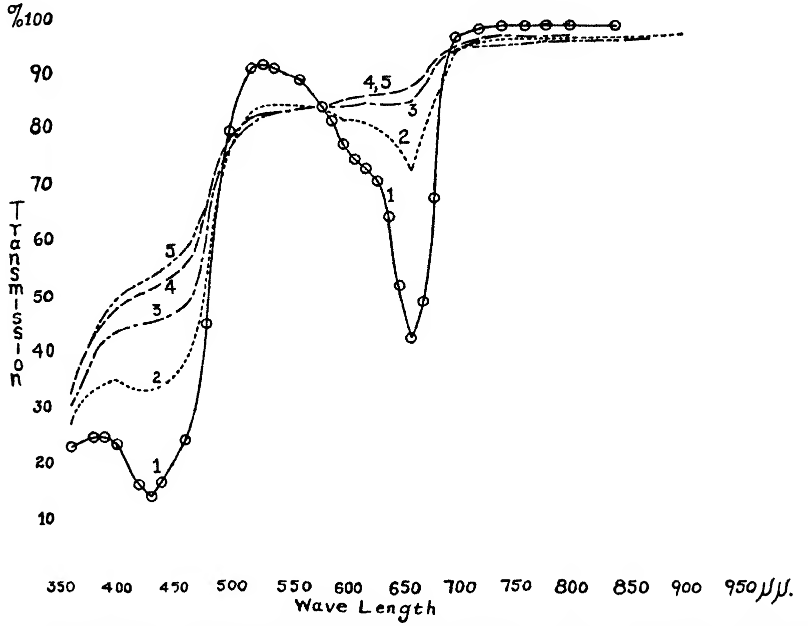

FIG. 1. Comparative curves showing effects of irradiation upon an alcoholic leaf extract.

That light is the factor causing the disintegration of the solution of leaf green was indicated by tests made on an ethyl alcohol solution of leaf extract maintained in the dark for a two day period. The results obtained from this test showed such slight changes that they could not be noticed in a curve having the same scale as figure 1.

COLLEGE OF WILLIAM AND MARY
VIRGINIA POLYTECHNIC INSTITUTE, NORFOLK DIVISION
AND
U. S. PUBLIC HEALTH SERVICE
CRANEY ISLAND LABORATORY

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HEXOSE-PHOSPHATE IN ALFALFA HAY

S. H. Thompson, Jr., and W. E. Tottingham

In a search for inosite hexa-phosphate in alfalfa hay one of us (**2**) recovered a preparation which appeared to be a sugar phosphate. The recent identification of hexose-phosphates in the sugar beet and pea plants by Burkhard and Neuberg (**1**) and Hassid (**3**) respectively, has renewed interest in the early result.

Samples of field-cured alfalfa hay were examined from the first cutting in the third season. These were produced on a well limed clay loam deficient in potassium, with application of potassium chloride, either alone or in addition to superphosphate.[1] Each sample was separated essentially into leaf blades and stems, the latter fraction carrying most of the petiole tissue. Lipides were extracted from the ground tissue with boiling 85 per cent. ethanol, which should have inactivated phosphatase (**1**). Subsequent procedures were based on experience of a former associate[2] with yields and stabilities of phosphorus fractions from soybean leaves.

The tissue was subsequently extracted with 2.0 per cent. acetic acid for two hours at about 12° C. Filtration, washing, neutralization, and fractionation of the extract by means of lead were also conducted at the lowered temperature. Large proportions of both inorganic and organic phosphorus were precipitated by neutral lead acetate. The latter fraction was neglected, however, owing to limitations of time and anticipation of more certain results from the basic lead precipitate. Only traces of the total phosphorus of the stem occurred in this basic fraction in any sample and also in the leaf of the unfertilized crop. Leaves from the K-fertilized and K,P-fertilized crops contained 1.6 and 2.6 per cent., respectively, of the total phosphorus in the basic lead precipitate.

The lead salts were decomposed by H_2S and the hexose-phosphate was converted to calcium salt and monophosphate osazone by the procedures of Neuberg (**4**) and Robison (**5**). It was infeasible to obtain the melting point of the low yield of osazone from the unfertilized hay but this function gave values of 140.3° and 140.0° for the other samples. The results agree closely with Robison's value of 139°. Our results appear to substantiate the occurrence of hexose-monophosphate in alfalfa hay, which thus survives the action of phosphatase in the curing process.

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[1] We are indebted to Professor C. J. Chapman of this institution for providing these samples.

[2] Dr. H. W. E. Larson, the University of Idaho.

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NOTES

Seventeenth Annual Meeting.—The seventeenth annual meeting of the American Society of Plant Physiologists was held at Philadelphia from December 30, 1940, to January 1, 1941. The programs were unusually valuable, and the attendance at some of the joint sessions broke all records. The symposium of Monday, December 30, on *The Structure of Protoplasm* was without question the best symposium we have had in years. Nine papers were presented, covering many of the most difficult phases of protoplasmic structure. The papers were masterful throughout, and worthy of a permanent place in the literature of plant physiology. The success of this symposium illustrates what can be accomplished by careful planning and integration of the discussions. Including the joint sessions, the program carried 82 titles, and all sessions drew attentive audiences.

The annual dinner was held in the Betsy Ross room of the Benjamin Franklin Hotel on Monday evening, December 30, with more than 180 present. The features were the CHARLES REID BARNES life membership award, the STEPHEN HALES award, and the sixth STEPHEN HALES address by Dr. JOHN W. SHIVE. His subject was *Significant Rôles of Trace Elements in the Nutrition of Plants.* This paper also served as his retiring president's address. It was an exceptionally clear presentation of some of the interrelationships of such elements as boron and calcium, manganese and iron, etc., in the metabolism of plants. We hope to present this paper later to all members in the pages of PLANT PHYSIOLOGY.

To the officers of the Society, and especially to the program committee, composed of Dr. WILLIAM SEIFRIZ, of the University of Pennsylvania, chairman; Dr. J. T. CURTIS, University of Wisconsin; Dr. W. R. ROBBINS, the New Jersey Agricultural Experiment Station; and the secretary-treasurer, Dr. W. E. LOOMIS, Iowa State College, are extended sincere congratulations and thanks for the unusual excellence of every phase of the program and meeting. It is not an accident when meetings are of unusual value and significance, but a reflection of the energy, skill, and judgment which are brought to the program tasks. Reports of special phases of the dinner program are contained in subsequent notes.

Life Membership Award.—The seventeenth CHARLES REID BARNES life membership award was made to Dr. WILLIAM FRANCIS GANONG, who has been Emeritus Professor of Botany at Smith College, Northampton, Mass., since 1932. The choice made by the committee, headed by Dr. H. R. KRAYBILL, Purdue University, is a very happy one, and reflects great honor upon the society. Dr. GANONG was born at St. John, New Brunswick, February

19, 1864; he received his B.A. at New Brunswick in 1884, M.A. in 1886, A.B. at Harvard in 1887, and Ph.D. at Munich in 1894. He also received a Ph.D. at New Brunswick in 1898, and LL.D. in 1920. He became assistant in botany, 1887–1889, and instructor, 1889–1893, at Harvard University. In 1894 he became Professor of Botany and Director of the Botanic Garden of Smith College, a position which he held for 38 years, until his retirement in 1932. He was elected second president of the Botanical Society of America in 1908, and is a corresponding member of the Royal Society of Canada. Dr. GANONG was an excellent teacher, a very exacting and careful laboratory technician, inventor of many devices for physiological experiments, and a writer of textbooks and manuals which exerted a profound influence upon the teaching of plant physiology.

He and Dr. BARNES were contemporaries, BARNES about 5 years older, and they were good friends. It is very gratifying to have Dr. GANONG added to the list of CHARLES REID BARNES life members.

It was at Philadelphia in 1926 that the first award was made, to Dr. BURTON E. LIVINGSTON, of Johns Hopkins University. Since that time one or more awards have been made each year. Of the 17 recipients, 14 are living at the present time.

Stephen Hales Award.—The seventh recipient of the STEPHEN HALES award is Dr. PHILIP RODNEY WHITE, of the Rockefeller Institute for Medical Research, Princeton, New Jersey, for his work on the tissue culture of excised roots. Dr. WHITE was born in Chicago in 1901, and received his higher education at Montana (A.B. 1922); the University of Washington (1922–1923); Ecole nor. d'Inst. Valence, France, certificat, 1924; and Johns Hopkins University (Ph.D. 1928).

After short service as micro-technician in the Bureau of Plant Industry, 1925–1926, and as special investigator for the United Fruit Co., 1926–1928, he became assistant professor of botany at the University of Missouri in 1928. He was a National Research Council fellow at the Boyce Thompson Institute in 1929–1930, and Rockefeller Foundation fellow at the Pflanzenphysiologische Institut at Berlin, 1930–1931. Since 1932 he has been connected with the Rockefeller Institute for Medical Research, where his work has attracted wide and favorable attention. Many of his important papers have been published in PLANT PHYSIOLOGY. In 1938 he received the annual prize of the American Association for the Advancement of Science for work in a collateral field.

It is a pleasure to extend to Dr. WHITE the congratulations and felicitations of his colleagues and friends who are happy to see him receive this well deserved honor.

Corresponding Member.—The American Society of Plant Physiologists has elected to corresponding membership, Dr. P. BOYSEN JENSEN, Professor of Plant Physiology at the University of Kopenhagen. He is probably best known to American plant physiologists for his *Wuchsstofftheorie und ihre Bedeutung für die Analyse des Wachstums und der Wachstumsbewegungen der Pflanzen,* which was published in 1935, and made available in English by AVERY and BURKHOLDER in 1936. In 1938 he also published a textbook of plant physiology in Danish. This work, translated into German by MATTICK, was republished in 1939, and is a very fine statement of the fundamental principles of plant physiology. He has contributed to the literature of the subject for more than thirty years, and first proved the existence of growth hormones in plants. His American friends and admirers extend most cordial greetings and good wishes to him and his institution, and welcome him as a corresponding member.

Amendments.—A few small changes in the constitution of the American Society of Plant Physiologists have become desirable. These were considered and approved by the executive committee at Philadelphia; the proposals will be submitted by ballot from the secretary-treasurer's office for a vote on adoption, sometime during 1941. It is hoped that all members will be sufficiently interested to cast a ballot for or against the changes when they are submitted.

Monographs.—For many years the American Society of Plant Physiologists has desired to launch the publication of a series of monographs. The major problem has always been financial; but it now seems possible that a start may be made with relatively small risks, if members will cooperate in the plan. The secretary-treasurer expects to be able to lay a proposition before us that will involve mainly pre-publication subscriptions at reduced prices. If the protoplasm symposium could be published at a cost of $2.00, for instance, and pre-publication subscriptions for 200 copies could be obtained, the remainder of the risk might be floated in some other manner not involving the society's finances. In case such proposals should come in concrete form, it is urged that a generous response be manifested. There is nothing we cannot do, if we desire it with our purse strings open. Success with the first venture would make succeeding ones easier to finance.

Galleys.—A warning to all contributors to PLANT PHYSIOLOGY is in order, that when galleys are unduly delayed, they will be set aside for the succeeding number. We now have reached the condition in which the journal can appear on time if the delay of slow proof reading can be eliminated. Receipt of a set of galleys is a demand that other affairs be laid aside until they are again in the mails. Those who respond most promptly will be the ones who

appear in the next number, and the slow ones will be left for three months later. This is to avoid the nullification of the editorial speed by those who hold up paging by long delays in forwarding corrected galleys.

Southern Section.—The Southeastern Section of the American Society of Plant Physiologists petitioned for a change in territory to coincide with that of the Association of Agricultural Workers, with which it meets, and for a change in name to Southern Section. These changes were granted at the Philadelphia meeting. The Southern Section will meet at Atlanta, Georgia, February 5 to 7, 1941. On February 6, a breakfast for the group will be followed by an organized round table discussion led by Dr. I. E. MILES, of Raleigh, North Carolina, on the general aspects of plant nutrition. All meetings will be held at the Piedmont Hotel. Any other information about the meetings may be obtained from the secretary of the section, Dr. T. J. HARROLD, University of Georgia, Athens, Georgia.

Necrology.—Several deaths have occurred among the members of the American Society of Plant Physiologists, which represent distinct losses to American science. The following brief biographies are memorials to those who have gone.

CARL LUCAS ALSBERG

Born in New York April 2, 1877, died in Berkeley, California, October 31, 1940. Dr. ALSBERG was the son of MEINHARD ALSBERG and BERTHA BARUCH ALSBERG. He was one of the early leaders in biochemistry, particularly as related to agricultural products. His early education was obtained at Columbia University, and the College of Physicians and Surgeons of that institution. He received the A.B. in 1896, A.M. and M.D. in 1900. Between 1900 and 1903 he spent time abroad at the universities of Strassburg and Berlin. In 1902 to 1905 he was assistant in physiological chemistry at Harvard, and from 1905 to 1908, instructor in biological chemistry at Harvard University. In 1908 he was selected to become chemical biologist with the bureau of plant industry in the U. S. Department of Agriculture, but in 1912 was transferred to the bureau of chemistry as chief of the bureau, a position he filled for nine years.

In 1921 he went to Stanford University as director of the Food Research Institute, and finally in 1937 he was invited to become director of the Giannini Foundation of Agricultural Economics at the University of California. This position he filled at the time of his death.

He was a member of many scientific organizations, and had served as officer and editor for several of them. As a result of his contacts and study, he had broad general grasp of agricultural social and economic problems.

His earlier scientific interests had included the nucleic acids, proteins, enzymes, toxicology, and cyanogenesis. One of his later contributions, which will be remembered by all of us, was his interesting discussion of the intimate structure of the starch granule, published in PLANT PHYSIOLOGY **13**: 295–330. 1938.

FRANK MARION ANDREWS

Last spring the April number of PLANT PHYSIOLOGY was dedicated to FRANK MARION ANDREWS in honor of his approaching 70th birthday anniversary. He was ill at the time, but it was hoped that he might regain strength enough to enjoy his retirement as emeritus professor at Indiana, which occurred in May. He had made considerable progress, but shortly after the Thanksgiving season, he took cold, which developed quickly into pneumonia. In his weakened condition, he was not able to overcome it, and died on November 26, 1940.

The biographical sketch published in the April number gives sufficient details of his work and life, so that nothing more needs to be added now. He is survived by his widow, MARIE O. ANDREWS, and to her we extend the sympathy of all members of the American Society of Plant Physiologists.

CAROLINE SHELDON MOORE

Miss MOORE was born at Kewanee, Illinois, February 17, 1871, died at Redlands, California, May 21, 1940. She was educated at Chicago (A.B. 1897), and spent short periods at Washington, and Oxford. Her early experience in teaching was in academies in Wisconsin (1896–1899), and Illinois (1899–1901). She was connected with Mt. Holyoke College in 1901, and remained there until 1905. From 1906 to 1915 she was engaged in private teaching and social service work. She then went to Linfield College, McMinnville, Oregon, where she spent six years. She had been associate professor of biology at the University of Redlands since 1921. Her main interest, as stated by herself, was the desmids of San Juan Islands.

Symbiotic Nitrogen Fixation.—A monograph entitled The Biochemistry of Symbiotic Nitrogen Fixation has been published by The University of Wisconsin Press. The author is Dr. PERRY W. WILSON, associate professor of agricultural bacteriology at the University of Wisconsin. It is a work of 302 pages, contains 34 plates, many additional text figures, and the discussions are presented in eleven chapters, which cover the subject in admirable fashion. The first chapters are general, and deal with the nitrogen economy of man and nature; and leguminous plants in agricultural history. Other chapters concern the biochemistry of bacteria; interaction of host and bacteria; fixation of nitrogen by bacteria and plant; the carbo-

hydrate-nitrogen relationship in nitrogen fixation; excretion of nitrogenous compounds by legumes; the chemical mechanism of the fixation process; physical-chemical characteristics of the enzyme system; practical applications; and concluding observations. It summarizes the literature of nitrogen fixation, which occupies 32 pages of citations. This monograph is highly recommended to anyone interested in the permanence of agriculture and nitrogen fixation research. It is well written, and may be obtained from the University of Wisconsin Press, 811 State St., Madison, Wisconsin, at $3.50 per copy. Libraries also must have it as a summary of the far-flung literature.

Methods of Enzyme Research.—The BAMANN-MYRBÄCK monograph on *Die Methoden der Fermentforschung,* notice of which was given in the October, 1940, number of PLANT PHYSIOLOGY, is coming from the press of George Thieme, Leipzig, very rapidly. Lieferungen 2, 3, and 4 have already come from the press. No. 2 contains 204 pages; no. 3, 392 pages; and no. 4, 408 pages, bringing the total issued to date to 1276 pages, or about a third of the entire work. The prices quoted for these three large sections are RM 22.80, 29.40, and 30.60 respectively, or RM 82.80 for all three. These are the foreign prices, and are not subject to discounts. It is not possible in a work of this kind to go into detail as to the contents. They must prove enormously valuable to all who wish to work in any field of enzyme research. Lieferung 2 continues the section on carbohydrates and their derivatives, and takes up nucleic acids and their derivatives; proteins and their derivatives; the amides; acceptor chromogens; thiols and disulphides; conjugated materials; and begins consideration of substrates. Lieferung 3 takes up such techniques as the determination of the constitution of high molecular weight compounds by use of enzymes, roentgenology, absorption spectra, Raman spectra, fluorescence, polarography, magnetics, viscosity measurements, ultracentrifuge, dielectrics, cryoscopy, osmotic methods, melting points, and sublimation. The last part of Lieferung 3 begins the consideration of enzymes, nomography, control of conditions, redox potentials, and the calculation of the free energy of biochemically important reactions. The fourth Lieferung continues with the methods of following enzyme effects, first the physical and physico-chemical methods, then the chemical methods, and biological methods. There is also a section on enzymic histochemistry.

It then takes up the preparation and testing of enzyme preparations: dissolved enzymes, active cell preparations, especially from bacteria. This subject continues in the following Lieferung 5, which has not been seen.

The work is most highly commended to all workers in these fields, and research libraries should consider it a "must" item. It is hoped that the remainder of the work can be issued expeditiously, so as to be available to all investigators engaged in enzyme research.

Temperature.—An interesting monograph, *Life and Death at Low Temperatures*, by Dr. BASILE J. LUYET and P. M. GEHENIO of Saint Louis University, comes as the first of a series of monographs in the field of general physiology, edited by Dr. LUYET, and published by *Biodynamica*, at Normandy, Missouri. This work does not attempt to cover the field of cold-hardiness in plants, nor the refrigeration of biological materials.

It is divided into three sections: (I) The lower limit of vital temperatures; (II) the physical states of protoplasm at low temperatures; (III) the mechanism of injury and death by low temperature.

Part I covers the field of lower limits for infracellulars, such as vitamins and hormones, enzymes, enzymoids and viruses; unicellular plants and animals; germ cells, spores and seeds; isolated cells and tissues of both plant and animal origin; and the higher metaphyta and metazoa.

The second section has a preliminary chapter on the fundamentals of heat conduction, and three chapters: on freezing, the frozen state and melting; supercooling and the supercooled state; and the vitreous state, vitrification, devitrification and vitromelting. This section will remind Dr. LUYET's many friends of the fascinating demonstrations that he has presented before his colleagues at scientific meetings.

The last part presents chapters on the action of cold without ice formation; and action of cold accompanied by ice formation.

The general bibliography, chronologically arranged and beginning in 1736, occupies 33 pages. A subject index and author index add to the facility of use.

It is a stimulating work, worthy of attention by plant and animal physiologists, whether or not one always agrees with the interpretations. Certainly the results recorded in the sections on vitrification are a distinct and valuable contribution to our knowledge of low temperature behavior of living matter. The fact that considerable proportions of it are reprinted from *Biodynamica* does not detract from its value.

It contains 341 pages, 33 illustrations, and can be purchased from *Biodynamica* at $4.50 per copy.

JETHRO TULL
1674–1740

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JETHRO TULL

IN MEMORIAM

CHARLES A. SHULL

(WITH ONE PLATE)

The two-hundredth anniversary of the death of JETHRO TULL occurred in the early days of March, 1940, the exact date being unknown; he was buried on March 9, 1740, at Basildon, Berkshire, England, amid such quiet and lack of publicity that no public record of his death and burial place was made. This anniversary should not be allowed to pass by without refreshing our memories of the man, who, more than any one else, was responsible for the development of genuine cultivation of the soil to improve its fertility. A celebration of the 200th anniversary of the publication of his book was held at the 10th annual meeting of the American Society of Plant Physiologists in 1933. The writer's address on that occasion was not published because it was an illustrated, informal discussion. This memorial presents the main facts of TULL's life as brought out in the address before the Boston meeting.

JETHRO TULL stands out as a great leader in an age of ignorance concerning the productiveness of the earth. As a contemporary of STEPHEN HALES, he shares the honor of leading in the development of new ideas. HALES was the more scientific, TULL the more practical; one foreshadowed the development of the fundamentals of plant physiology, the other the fundamentals of practical utilization of soils and crops for human welfare. Both deserve a full measure of praise from present day scientists whose work continues, deepens, and strengthens the principles discovered by these pioneers.

There were other writers in the agricultural field before JETHRO TULL, of course. The following are a few of them: FITZHERBERT, author of *The Boke of Husbandrie,* in 1534; RICHARD WESTON, with his *Discourse of Husbandry as Used in Flanders,* 1645; JOHN EVELYN's *Terra,* 1658; and

JOHN MORTIMER's *Whole Art of Husbandry*, 1707. These are the better known observers and recorders previous to TULL's time.

It was JETHRO TULL's belief in the soil, his originality and independent thinking, his keen observations, his intuitive perception of causes and effects, and his courageous industry in the face of great trials and difficulties that made him a great agricultural leader during the first four decades of the eighteenth century. It was undoubtedly accidents of life, inheritance or acquisition of property, that led him away from legal, political, and cultural pursuits, and to a life of struggle with the forces of nature, and to a struggle against human inertia, stubborn ignorance of the common people, and the persecutions of his detractors and defamers. The resistance of laborers against his innovations in practice, and his ingenious inventions, which required them to perform unwonted tasks, is a revealing commentary on the times.

These laborers were insolent, disobedient, and hated the new practices and the new tools. The tools were often broken surreptitiously by some sullen workman, to make them look impractical; or parts were thrown into the well so that they need not be used. Careful directions for seeding and culture were obstinately disobeyed; or plants deliberately destroyed to prevent success with the experiments. If you wonder why he didn't fire such workmen, as we would today, it is explained by the laws which were designed to protect these ignorant laborers from injustice. The landlord was practically owned and run by the workers, who could not easily be dispossessed of their means of livelihood for their malpractices.

Many nasty epithets were used, to make TULL look ridiculous. After his book appeared his detractors tormented him with scurrilous criticisms. One group of these, a society headed by one STEPHEN SWITZER, had an anonymous writer who called himself "Equivocus." TULL called the whole set "The Equivocal Society." Earl CATHCART quotes TULL's comments on these tormenters as follows: "The Secret Society—the Equivocal Society—likewise are not content with abusing my vegetable principles, and terming me an atheist, but also describe me by a similitude of the most odious, despicable, and pestiferous animals. They also usurp the power of the Inquisition in damning books because not their own. And, again, they, the critics in question, seldom make use of any other logic than that of Billingsgate; they call me names—atheist, infidel, fool, mente captus, madman, ass, owl, viper, carping insect, &c, all feminine arguments of scurrility." Time has put these braying asses where they belong, and TULL's principles are immortalized in all of the arable lands of the earth.

Not only was TULL beset by low-browed workers and critics; he was ill most of his life, with kidney stones, and other equally painful and at that time incurable diseases. He developed a chest ailment for which he had

to spend several years in sunny Italy. In spite of pain and disease his mind was ever at work, thinking up new practices, new instruments, plows, harrows, drills, cultivators, hoes, etc., to give more effective nourishment to crops, more even stands of grain, to use less seed, to secure better germination, and a better, more fertile soil.

His home life must not have been very congenial. His wife was probably not overly sympathetic with her suffering husband; and his only son turned out to be a ne'er-do-well. He had no one to turn to in those moments when he most needed understanding and faithful, human companionship.

It was in the midst of such harassments that his great book, *The Horse Hoeing Husbandry*, was written. He had to depend upon a scribe for the preparation of his manuscript from his notes; and the scribe was not careful enough to get things down in the right order. The publishers, also, were not sufficiently cooperative with the proofs and changes before the work was actually printed. TULL explains all of these difficulties in his preface, and gives a clear picture of the tremendous odds against which he struggled. It was at the urging of many really interested and notable personages, who visited him at Prosperous Farm, and who saw the true value of TULL's experiments that he at last consented to make a permanent record of his ideas, discoveries, and inventions.

Turning now to his life story, which may be told very briefly, he was the son of JETHRO and DOROTHY TULL. His father, JETHRO, SR., came from Midgham, in the Kennet valley, between Newbury and Reading; and his grandfather, GILES TULL, had been the church warden at Midgham in 1641.

His father and mother moved to Basildon, in Berkshire, and were living there when JETHRO, JR. was born, sometime in March, 1674. The church records show that JETHRO was baptized on March 30, 1674.

Little is known of his boyhood; but when he was 17 years old he went to Oxford, where he matriculated in St. John's College on July 7, 1691. He did not finish the course at Oxford, and a couple years later he was admitted as a law student at Gray's Inn, London, December 11, 1693. He also spent two years at Staple Inn. He had hoped to make his contribution to political life, and was interested mainly in the principles of the English constitution, not in the practice of law.

After his student days had ended, he made a grand tour of the continent of Europe. This was sometime between 1693 and 1699, and he took careful notes on the soils, methods, implements, vegetable productions, and any agricultural practices employed in the lands he visited. The culture of vineyards in France was especially enlightening, and he was struck with the idea of applying similar deep culture to all agricultural crops.

He was admitted to the bar on May 19, 1699, after his return from his continental travels; and in the autumn of that year, October 26, 1699, was

married to SUSANNA SMITH of Burton-Dassett, Warwickshire. There were five children born to them, one son and four daughters.

Their first home was at Howberry, Crowmarsh, just across the Thames from Wallingford in Oxfordshire. Here JETHRO engaged in farming, and invented many of his most useful tools. The drill is believed to have been invented about 1701.

After almost a decade of hard labor at Howberry, he moved to Prosperous Farm in Berkshire. Shortly thereafter he spent three years, 1711 to 1714, in Italy, as has been mentioned. On his return to Prosperous Farm, he made of it a living demonstration of his practical ideals. Many notables visited him, and carried away new hope for agriculture in the British Isles. The new culture was taken up in Scotland, and wherever it was diligently applied, great improvement of agriculture resulted. It was only the importunity of these interested people who saw beyond the horizons of the day that finally induced TULL to take up the painful and distasteful duty of writing his book.

He lived at Prosperous Farm, in the southwestern corner of Berkshire, for 26 years, until his death, in 1740. So little attention was paid to his passing away that no public record was made of his burial place; and for nearly 150 years his last resting place remained unknown. But JETHRO TULL's fame, shining ever more brightly out of the darkness of the early eighteenth century finally challenged intelligent and devoted research to locate his burial place. In 1889, after 20 years of patient search, the problem was solved by WALTER MONEY, who discovered the church record of his burial in the Parish Register of Basildon, which reads: "JETHRO TULL gentleman of the Parish of Shalburne was buried March ye 9th 1740. Mem. This JETHRO TULL Esquire was the author of a valuable book on agriculture, entitled Horse Husbandry. GEO: BELLAS, Rector." GEORGE BELLAS was vicar at Basildon at the time of TULL's death and burial, and rector of the neighboring Parish of Yattenden.

Thus the life of this great man is marked at the beginning and the end, not by the actual dates of birth and death, but of baptism, and burial. In the light of our day, TULL stands out as one of the really great benefactors of mankind, whose principles now form the basis of all intelligent and successful cultivation of the earth, and of man's escape from the gloomy predictions of MALTHUS. The breaking of the soil, the drilling of the seed, the fruitful yields of the fertile earth form a perpetual and enduring monument to his genius. May we never forget how much we owe him.

THE UNIVERSITY OF CHICAGO

INHIBITION OF INCREASE AND ACTIVITY OF TOBACCO-MOSAIC VIRUS UNDER NITROGEN-DEFICIENT CONDITIONS

ERNEST L. SPENCER

(WITH ONE FIGURE)

Introduction

Although viruses cause many plant diseases, little is known regarding their metabolism since they apparently multiply only in living tissue. Here it is difficult to differentiate between the metabolism of a host and that of a pathogen. Multiplication of viruses *in vivo* is a well recognized fact and thermal inactivation *in vivo* of a few viruses has been definitely established. No other method for the inactivation of virus *in vivo* has been demonstrated experimentally.

In this study on virus metabolism, a knowledge of the nitrogen metabolism of normal tobacco plants and the discovery that nitrogen supplied to a virus-infected plant had an effect on virus concentration (**19**) have made it possible to distinguish to some extent between the anabolic and katabolic processes involved. It is well known (**14, 24**) that in mature leaves of a nitrogen-deficient plant proteins are hydrolyzed and proteolytic decomposition products liberated. These are translocated to the growing point where they supply the meristem with materials necessary for protein synthesis. A study of the effect of withholding nitrogen from a tobacco plant diseased with tobacco mosaic on the total quantity and specific biological activity of virus within the plant has thrown light on the following questions: Is the virus a stable entity under conditions of nitrogen deficiency, or is it subject to normal protein hydrolysis with a subsequent loss in either amount or activity or both? If the virus is hydrolyzed, can the proteolytic products then be used in meeting the normal nitrogen requirements of the plant, or can they be reassembled into fully active virus? A series of experiments were carried out in an effort to determine in what way tobacco-mosaic virus is affected in a nitrogen-deficient plant. Such studies have been made possible by a combination of the techniques of growing plants in nutrient culture, purification of virus by high-speed centrifugation, and estimation of virus activity by a modification of the local lesion method. The results of the studies, a partial summary of which has been published (**20**), are reported in this paper.

Experimental procedure

Turkish tobacco plants (*Nicotiana tabacum* L.) were used as host plants in all experiments. Uniform seedlings in the 3- or 4-leaf stage were trans-

planted into 4-inch porous clay pots filled with washed, white quartz sand. The pots were placed in clay saucers on a bench in a greenhouse, the temperature of which was held between 70° and 80° C. most of the time. Three days after being transplanted, the plants were inoculated with fresh juice from a tobacco plant diseased with tobacco-mosaic virus (*Marmor tabaci* H.).[1] Nutrient treatments were started at time of inoculation. Each plant received 100 ml. of a complete nutrient solution every second day and water between nutrient applications whenever necessary. The nitrogen level of the solution (200 p.p.m. of nitrogen) had been found in previous tests to produce normal growth comparable to that obtained in a rich composted greenhouse soil. The composition of the solution, together with that of a solution deficient only in nitrogen, reference to which will be made later, is given in table 1. In addition to the salts listed in the table, each solution

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS

NUTRIENT SOLUTIONS	VOLUME OF 0.5 MOLAR STOCK SOLUTIONS PER LITER OF NUTRIENT SOLUTION				
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	$(NH_4)_2SO_4$	$CaCl_2$
	ml.	*ml.*	*ml.*	*ml.*	*ml.*
Complete	12.9	11.0	4.0	3.3	0.0
Minus nitrogen	12.9	0.0	4.0	0.0	11.0

was supplemented with ½ p.p.m. of both boron and manganese as H_3BO_3 and $MnSO_4$, respectively.

At intervals, representative plants were cut, weighed, and then placed in covered pans in a cold room held at – 14° C. Twenty-four hours later the frozen plants were minced in a food chopper. The pulp was mixed with 3 per cent. by weight of K_2HPO_4 and allowed to thaw at room temperature. The cold juice was expressed from the pulp through cheese cloth by means of a small screw press and cleared of all extraneous insoluble materials by low-speed centrifugation. Aliquots of this juice were then assayed for virus activity, total protein, and total virus protein. The assay of plant proteins included all soluble protein fractions. No account was taken of the insoluble fractions.

Virus activity of juice from diseased plants was assayed by means of the local lesion method (**4**), using bean (*Phaseolus vulgaris* L. var. Early Golden Cluster) as the test plant (**16**). Although the use of this method does not give a measure of the absolute amount of active virus present, certain modifications have recently been made by means of which it is possible to esti-

[1] Latin names of viruses used in this paper were taken from the Handbook of Phytopathogenic Viruses (5).

mate, usually within 10 to 20 per cent., the quantitative difference in virus activity between two or more samples. Data on this modified method and experiments pertaining to its accuracy will be published later (**22**).

Total soluble protein content of juice was determined by precipitation of the protein by hot trichloroacetic acid. One ml. of juice was treated with 1 ml. of hot 5 per cent. trichloroacetic acid and immediately cooled. Following centrifugation, the denatured protein was dissolved in 1 ml. of 0.2 N NaOH, reprecipitated with 1 ml. of 10 per cent. trichloroacetic acid and again centrifuged. The protein precipitate was transferred to a micro-Kjeldahl flask with 1 ml. of 0.2 N NaOH and analyzed for Kjeldahl nitrogen by the modified method (**11, 15**) of FOLIN and FARMER (**3**), as follows: To the protein solution was added 2 ml. of concentrated H_2SO_4, 1 gm. of K_2SO_4, 2 drops of SeOCl, and a few alundum chips. The mixture was digested for at least 5 minutes after the contents had become clear or straw-colored. After the addition of 30 ml. of water and 6 ml. of 50 per cent. NaOH, nitrogen as ammonia was distilled over into 0.02 N HCl. All titrations were made with 0.02 N NaOH, using methyl red as an indicator. This determination included both soluble plant protein and virus protein.

The virus protein was isolated by means of an air-driven ultracentrifuge as previously described (**21**). Stainless steel centrifuge tubes were used in place of thin-walled celluloid tubes. The content of sedimented virus, as measured by the virus-protein content, was then assayed by digestion of the virus protein with H_2SO_4, K_2SO_4, and SeOCl, as outlined above.

This experiment was carried out during September and October. Similar experiments conducted during November and December and again during early summer gave comparable results.

Results

A study was first made of the effect of nitrogen starvation on young plants showing severe systemic symptoms of tobacco mosaic. Starting at time of inoculation, all plants were fed the complete nutrient solution. Ten days after inoculation the plants were divided into two groups. One group continued to receive the complete nutrient solution every second day. The other group, after a thorough flushing of the sand to remove as much nitrogen as possible, was fed the minus-nitrogen solution every second day. At 4-day intervals, representative plants from each group were harvested and analyzed for total protein, virus protein, and virus activity.

In table II are presented data pertaining to the growth of diseased plants that received the two nutrient treatments. Four days after transfer, the nitrogen-fed plants weighed about 15 per cent. more than those deprived of nitrogen. With each succeeding harvest thereafter, the difference in size of plants between the two groups became progressively greater, until by the

24th day the nitrogen-fed plants were nearly 5 times the size of those deficient in nitrogen. During this 24-day period, the green weight of nitrogen-fed plants increased about 18-fold, whereas that of nitrogen-deficient plants increased less than 4 times. A portion of this increase in nitrogen-deficient plants was probably due to the reserve of unassimilated nitrogen present in these plants at the time they were transferred to the minus-nitrogen treatment.

Table II also shows the virus activity of juice from the nitrogen-deficient plants relative to that of juice from nitrogen-fed plants of the same age at

TABLE II

DATA ON GROWTH OF DISEASED TOBACCO PLANTS AND ON RELATIVE VIRUS ACTIVITY OF THEIR JUICES, WHEN HARVESTED AT INTERVALS AFTER TRANSFER TO THE MINUS-NITROGEN TREATMENT

TIME OF HARVEST: DAYS AFTER TRANSFER TO MINUS-N TREATMENT*	NUMBER OF PLANTS HARVESTED		AVERAGE PER PLANT				VIRUS ACTIVITY† OF N-DEFICIENT PLANTS PER	
			GREEN WEIGHT		VOLUME OF JUICE EXPRESSED			
	+N TREATMENT	−N TREATMENT	N-FED PLANTS	N-DEF. PLANTS	N-FED PLANTS	N-DEF. PLANTS	ML. OF JUICE	PLANT
days			*gm.*	*gm.*	*ml.*	*ml.*	%	%
0	20		2.9		2.0			
4	12	12	5.4	4.7	3.0	2.8	100	93
8	10	15	9.5	5.2	5.5	3.7	64	43
12	6	10	16.2	7.5	9.0	4.7	54	28
16	5	10	24.8	7.6	17.8	4.6	87	22
20	5	10	38.2	10.3	26.8	6.4	64	15
24	5	7	52.8	11.1	34.0	7.0	32	7

* Minus-nitrogen treatment started 10 days after inoculation.
† Virus activity relative to that of N-fed plants harvested at same time.

each of the 6 harvests. In each case the activity of the nitrogen-fed plants was assumed equal to 100 per cent. The virus activity per ml. of juice from plants on the minus-nitrogen treatment for 4 days was apparently equal to that of nitrogen-fed plants. After 24 days on the minus-nitrogen treatment, however, the juice from these plants was only $\frac{1}{3}$ as active on a unit volume basis as was that from plants receiving an adequate nitrogen supply. The last column in table II shows the calculated relative virus activity of the nitrogen-deficient plants at each of the harvests. These calculations are based on the volume of juice expressed per plant and the relative virus activity per ml. As shown by the data, the difference in virus activity between the two nitrogen treatments became greater and greater, until by the 24th day the nitrogen-fed plants had nearly 15 times as much virus as did nitrogen-deficient plants of the same age. Possible explanations for this large difference will be discussed in a later section.

Data pertaining to the total-protein content and the virus-protein content of aliquots of juice from the two sets of plants at each harvest are recorded in table III. In plants receiving nitrogen, the mg. of total protein per ml. of juice increased at first and then decreased slightly. The low protein contents recorded on the 16th and 24th day were probably due in part to the fact that each of these harvest days was preceded by one or more dark, cloudy days. The decrease in carbohydrate synthesis is accompanied by an accelerated hydrolysis of stored proteins. In nitrogen-deficient plants

TABLE III

CONTENT OF TOTAL PROTEIN AND CONTENT AND RELATIVE ACTIVITY OF TWICE-ULTRACENTRIFUGED VIRUS PROTEIN IN JUICE FROM DISEASED PLANTS HARVESTED AT INTERVALS AFTER TRANSFER TO THE MINUS-NITROGEN TREATMENT

TIME OF HARVEST: DAYS AFTER TRANSFER TO MINUS-N TREATMENT*	TOTAL PROTEIN (MG. PER ML.)		VIRUS PROTEIN (MG. PER ML.)		ACTIVITY† OF VIRUS PROTEIN FROM N-DEF. PLANTS: PER UNIT WEIGHT OF PROTEIN	ACTIVITY† OF N-DEF. PLANTS AS CALCULATED FROM VIRUS-PROTEIN DATA
	N-FED PLANTS	N-DEF. PLANTS	N-FED PLANTS	N-DEF. PLANTS		
days					%	%
0	11.1		2.7			
4	9.5	9.3	3.0	3.5	100	109
8	11.7	8.0	4.4	4.0	71	43
12	12.1	6.1	5.2	3.4	69	24
16	9.2	5.7	3.7	3.1	84	18
20	11.1	5.1	4.5	2.8	88	13
24	9.7	3.7	4.4	2.4	58	7

* Minus-nitrogen treatment started 10 days after inoculation.
† Virus activity relative to that of N-fed plants harvested at same time.

a steady decrease took place in the mg. of total protein per ml. This decrease was apparently brought about by a dilution of synthesized protein with subsequent growth and not to an actual loss of protein. Evidence substantiating this conclusion will be presented later. The virus data show that in nitrogen-fed plants the mg. of virus protein per ml. of juice increased up to the 12th day and then decreased slightly. This slight decrease may be due to an inadequate supply of nitrogen to insure a continuation of the same growth rate in such large plants. The mg. of virus protein per ml. of juice from nitrogen-deficient plants increased slightly at first and then decreased. As in the case of the total protein in these plants, the apparent decrease is only a dilution effect. The composite protein data show that at the last harvest 40 per cent. of the total protein in juice of the nitrogen-fed plants was virus protein, whereas in juice from plants starved for nitrogen, from 60 to 65 per cent. of the total protein was virus protein. This yield of virus is lower than that obtained by STANLEY (**23**), who reported a yield of crystalline virus equal to 80 per cent. of the total protein in the juice. No explanation for this discrepancy has been found.

The virus data discussed so far in table III indicate the virus-protein content in the juices from the two sets of plants but give no information regarding the activity of this virus protein. The fact that virus protein is not broken down does not necessarily imply that the virus is still active, for virus inactivation may not be synonymous with virus disintegration. As LAUFFER and PRICE have recently suggested (**10**), virus inactivation by heat is not identical with thermal denaturation of the virus but may be one of a series of reactions that eventually lead to denaturation. In their heat studies, carried out *in vitro*, virus inactivation proceeded at a faster rate than thermal denaturation.

The 6th column of table III records the biological activity on a unit weight basis of virus protein from the nitrogen-deficient plants relative to that of virus protein from plants receiving the complete nutrient solution. Four days after transfer, the activities per unit weight of virus from the two sets of plants were practically identical. Four days later, virus from the nitrogen-deficient plants was only 71 per cent. as active as that from nitrogen-fed plants. By the 24th day virus from nitrogen-deficient plants was only 58 per cent. as active on a unit weight basis. It might appear as though this value were unusually low in view of the values on the 16th and 20th day, but a later experiment indicated that these two values were not representative and that the 58 per cent. was approximately correct. As will be brought out later, this loss of nearly half the activity was not accompanied by any detectable denaturation or hydrolysis of virus even when nitrogen was withheld from the plant for 24 days.

In the last column of table III is shown the relative virus activity of the entire plant at each harvest, as calculated from the content of virus protein in the juice per plant and the relative activity per unit weight of this virus protein. These calculations show that after the first few days following the transfer from the complete nutrient to the minus-nitrogen treatment, the difference between the virus activities of the two treatments increased very rapidly. By the time the plants had been on the minus-nitrogen treatment for 24 days, juice from these plants was only 7 per cent. as active as that from the nitrogen-fed plants. It is worth noting that these values are essentially the same as those presented in the last column of table II. Although the same entity was measured in both cases, it is interesting that the two methods, one based on measurements with crude juice and the other based on measurements with purified virus, check each other so closely. In this connection it should be pointed out that only a small fraction of the difference between the virus activities of the two groups of plants was due to loss of activity of the virus on the part of the nitrogen-deficient plants. Most of the difference was due to an increase of virus in the nitrogen-fed plants.

In the experiments so far reported, a number of diseased plants was

transferred to the minus-nitrogen treatment 10 days after inoculation. In a second series of experiments, carried out in conjunction with those discussed above, tests were made with plants transferred to the minus-nitrogen treatment 18 days after inoculation. These plants were more than 3 times the size of those changed at the earlier date and were growing much more rapidly at the time of transfer. Representative nitrogen-fed and nitrogen-deficient plants were harvested every 4 days and assayed as in the first series of experiments. The analytical data obtained are recorded in table IV.

It is apparent from these data that the results obtained with the larger plants are very similar to those recorded with the smaller plants. The nitrogen-fed plants increased nearly 8 times in size during the 20-day period; the nitrogen-deficient plants little more than doubled in size. Size was measured by the volume of juice expressed, since this was roughly proportional to the green weight of the plants. The mg. of total protein and of virus protein per ml. of juice remained practically constant in the nitrogen-fed plants but decreased markedly in juice from the nitrogen-deficient plants. At the end of the experiment, 40 per cent. of the protein present in juice from nitrogen-fed plants was present as virus protein, whereas in the juice from nitrogen-deficient plants 55 per cent. of the total protein was accounted for as virus protein. The virus protein in nitrogen-deficient plants was only 70 per cent. as active after the 20-day period on the minus-nitrogen treatment, but no decrease in total mass of virus protein could be detected.

The virus-protein data so far considered have been based entirely on the mg. per ml. of expressed juice and, as such, give only a fragmentary picture of what took place within the plants. In order to fill in the picture, it is necessary to consider total yield of virus protein and of soluble protein per plant as extracted in the plant juice. The yields of virus protein and of total soluble protein in juice from plants on the two treatments at each of the several harvests are represented graphically in figure 1.

As shown by the upper line, the protein content of nitrogen-fed plants increased slowly at first while the plants were small, but as the plants became larger, the protein content increased more markedly. This indicated a rapid synthesis of proteins in these plants. A similar trend was shown by the virus-protein content in these plants as measured at 4-day intervals. The increase appears to be more gradual, whereas in reality the percentage increase was actually much greater. In the 24-day period during the test, the total protein increased about 15 times—from 22 mg. to 330 mg., whereas the virus protein increased 30 times—from 5 mg. to 150 mg.

The graphic representation of data pertaining to the protein content of plants transferred to the minus-nitrogen treatment 10 days after inoculation displayed an entirely different trend. After a small initial rise during

TABLE IV

VIRUS ACTIVITY AND PROTEIN CONTENTS OF JUICE FROM NITROGEN-FED PLANTS AND FROM NITROGEN DEFICIENT PLANTS AT INTERVALS AFTER INITIATION OF MINUS-NITROGEN TREATMENT, WHICH WAS STARTED 18 DAYS AFTER INOCULATION

TIME OF HARVEST: DAYS AFTER TRANSFER TO MINUS-N TREATMENT	NUMBER OF PLANTS HARVESTED		VOLUME OF JUICE EXPRESSED PER PLANT		TOTAL PROTEIN PER ML. OF JUICE		VIRUS PROTEIN PER ML. OF JUICE		ACTIVITY* OF N-DEFICIENT PLANTS PER		ACTIVITY* OF N-DEF. PLANTS AS ASSAYED WITH	
	N-FED PLANTS	N-DEF. PLANTS	N-FED PLANTS	N-DEF. PLANTS	N-FED PLANTS	N-DEF. PLANTS	N-FED PLANTS	N-DEF. PLANTS	ML. OF JUICE	UNIT WT. OF PROTEIN	CRUDE JUICE	VIRUS PROTEIN
days			*ml.*	*ml.*	*mg.*	*mg.*	*mg.*	*mg.*	%	%	%	%
0	10		5.5		11.7		4.4					
4	6	6	9.0	9.5	12.1	9.6	5.2	4.4	76	74	80	66
8	5	5	17.8	13.4	9.2	6.9	3.7	3.5	67	79	50	56
12	5	5	26.8	14.4	11.1	6.1	4.5	3.2	67	86	36	33
16	5	7	34.0	15.4	9.7	6.2	4.4	3.6	54	83	24	31
20	5	6	42.2	14.3	10.4	5.6	4.2	3.1	55	68	19	17

* Virus activity relative to that of nitrogen-fed plants harvested at the same time.

the first 8 days on the minus-nitrogen treatment, both the content of soluble protein and that of virus protein remained practically constant during the remainder of the experimental period of 16 days. These data show that the decrease in mg. of protein per ml. of juice, as recorded in table III, was due to a dilution of the protein already synthesized, with subsequent growth, rather than to a loss of this protein, since the protein content of the plant apparently remained constant. Graphs with a similar trend were found when the contents of total protein and virus protein in juice from plants

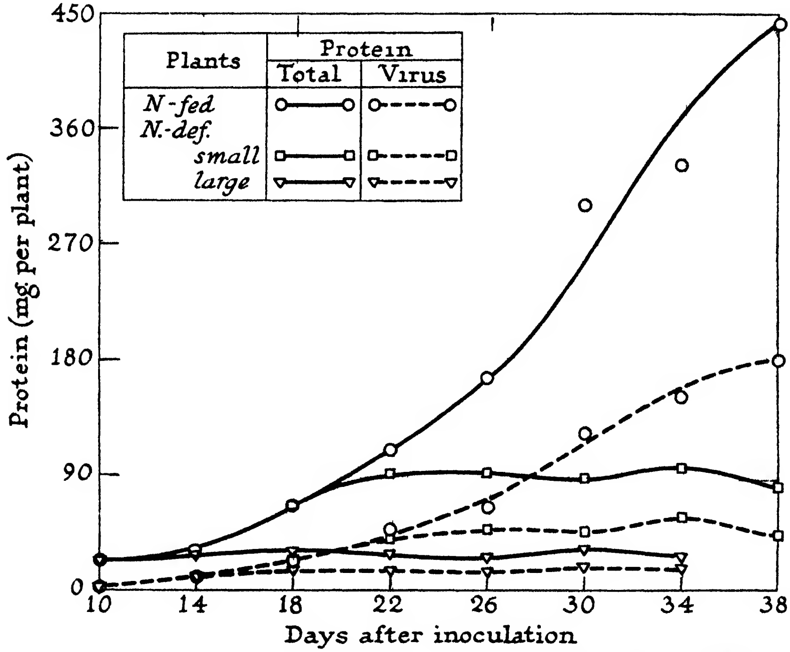

FIG. 1. Total protein and virus protein contents in juice from nitrogen-fed and nitrogen deficient plants at intervals following inoculation with tobacco mosaic virus.

held on the plus-nitrogen treatment for 18 days before being changed to the minus-nitrogen treatment were plotted.

From the 4 graphs in figure 1, obtained by plotting data from nitrogen-deficient plants, it is possible to draw several tentative conclusions regarding the nitrogen metabolism of a virus-infected plant. In the first place, even a plant suffering from severe nitrogen deficiency is apparently unable to utilize, in the synthesis of its normal proteins, any of the nitrogen in the virus, since its non-virus protein content does not increase and its virus-protein content does not decrease. Some change, however, does take place in the virus in such a plant to render it partially inactive without bringing about any detectable decrease in yield of virus protein per plant. On the

other hand, the virus is apparently unable to utilize any measurable part of the soluble proteins of the plant, for these constituents do not decrease. It is apparent, therefore, that the plant and virus may have competed against each other for the nitrogen absorbed by the plant. Such an hypothesis would explain why a mosaic-diseased plant shows symptoms of nitrogen deficiency at an earlier date than does a healthy plant grown under the same condition. Part of what little nitrogen is available in such plants would be utilized by the virus, rendering the deficiency condition even more severe.

Discussion

Several workers (**1, 13, 17, 23**) have suggested either directly or indirectly that in a mosaic-diseased plant normal proteins may be destroyed or utilized in the formation of virus. This hypothesis was not substantiated by observations in the present study in which it was found that the content of normal proteins in a diseased plant receiving no additional nitrogen did not decrease but remained more or less constant throughout the experimental test period. Although these observations were based on the soluble protein content of the expressed juice, the evidence showed that in the absence of an external supply of nitrogen no further virus multiplication could be detected. This would indicate that no conversion of soluble or insoluble normal proteins into virus took place under these conditions. Virus may multiply, however, at the expense of normal protein by utilizing in its metabolism some of the nitrogen absorbed by the plant before the plant can assimilate such nitrogen in the synthesis of normal proteins.

It has been reported (**13, 17**) that virus protein increases and then decreases with increasing maturity of the diseased plant. The evidence, which has been interpreted to show a destruction of virus, was based on the virus content per gm. of tissue. Similar results have been obtained (**6, 18, 19**) using crude juice. Data obtained in the present paper indicated the same trend, but when these data were calculated on a plant basis it was obvious that, in the absence of further multiplication, the virus content showed no decrease but remained constant. The above-mentioned papers do not contain sufficient growth data to permit such a calculation. The reduction per unit weight or per unit volume is apparently due to a dilution of the virus with subsequent growth of the plant and not to an actual loss or destruction of virus. The data in this paper show that the activity of the virus may decrease under certain conditions. Such an inactivation has not been demonstrated previously.

It is difficult to reconcile the results of the writer with those of Rischkov and Smirnova (**17**), who reported that, in tomato plants deficient in nitrogen, virus not only continued to accumulate but even reached a concentration equal to that present in plants supplied normal amounts of nitrogen.

The data presented here (table III and fig. 1) show a much higher virus content in nitrogen-fed plants than in nitrogen-deficient plants.

The partial inactivation of tobacco-mosaic virus in a nitrogen-deficient plant is interesting because of the relative stability of this virus *in vitro*. Two diseases of sugar cane, sereh disease of Java (**7**), and chlorotic streak (**12**), have been cured by heat treatment. The former is believed to be caused by a virus, but some doubt exists as to whether or not chlorotic streak is a virus disease (**2**). Recent experiments with such well recognized viruses as those of aster yellows (**9**), peach yellows, little peach, red suture, and peach rosette (**8**) have definitely established the fact that these viruses can be inactivated *in vivo* by heat. Ross (**18**) has recently reported a marked decrease with age of plant in virus activity of crude juice from tobacco plants diseased with alfalfa-mosaic virus (*Marmor medicaginis* H.). Since this decrease was too rapid to be due to a diluting effect of growth, he concluded that the virus was partially inactivated. No data were reported concerning the content and biological activity of sedimentable virus protein in these plants.

At present writing it is impossible to advance any definite explanation for the inactivation of virus *in vivo*. Ross (**18**) believes that the virus of alfalfa mosaic may have been inactivated by heat, since this virus has a low thermal inactivation point *in vitro* and since the virus never reaches a high concentration in tobacco plants grown during the summer months. Such an explanation would not hold in the case of tobacco-mosaic virus, as this virus is one of the most stable plant viruses so far studied, with a thermal inactivation point of about 90° C. If the virus is a living entity, it is possible that a portion may become avirulent or die out but not be disintegrated, thereby accounting for the loss in activity without a decrease in mass. If the virus is a protein molecule, its biological activity may be dependent on the presence of one or more active groups, and the loss or alteration of such a grouping may occur without a breaking apart of the macromolecule. It is apparent, however, that as far as the plant in a nitrogen-deficient condition is concerned, the virus protein acts as a foreign protein since it is not subject to normal proteolytic hydrolysis by the plant systems. Further work is necessary to elucidate the manner in which virus inactivation takes place in a nitrogen-deficient plant.

Summary

Experiments were carried out to determine in what way tobacco-mosaic virus is affected in a plant deficient in nitrogen. Turkish tobacco seedlings, grown in sand cultures and supplied a complete nutrient solution (nitrogen level = 200 p.p.m.), were inoculated with tobacco-mosaic virus. Ten days after inoculation the plants were divided into two groups; one group (nitro-

gen-fed plants) continued to receive the complete solution and the second group (nitrogen-deficient plants) received a nutrient solution complete in everything but nitrogen. Representative plants from each group were harvested at 4-day intervals and the expressed juice assayed for relative virus activity, total protein, and virus protein.

In nitrogen-deficient plants the virus-protein content as well as the content of soluble plant protein remained practically constant, whereas in nitrogen-fed plants each increased more than 5 times during a 16-day period. Although no decrease in the yield of virus protein in the nitrogen-deficient plants could be detected, its biological activity, however, decreased more than 40 per cent. It is not known how this inactivation was brought about.

As far as the nitrogen-deficient plant is concerned, the virus protein acts as a foreign protein, for the virus was apparently not affected by the normal proteolytic processes of the plant. Even a plant suffering from a severe nitrogen deficiency was unable to use, in the synthesis of its normal proteins, any nitrogen previously utilized by the virus. On the other hand, the virus was unable to utilize any nitrogen tied up in the proteins normally present in a nitrogen-deficient plant, for in the absence of an external supply of nitrogen, no further virus multiplication could be detected.

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RESPONSE OF SEEDLINGS TO VARIOUS WAVEBANDS OF LOW INTENSITY IRRADIATION[1,2]

ROBERT B. WITHROW

(WITH THREE FIGURES AND TWO PLATES)

The most extensive investigations concerning the influence of radiant energy within the visible spectrum on the growth of shoots of seedlings and storage organs have been conducted by COUPIN (**1**), MACDOUGAL (**2**), PRIESTLEY (**4**), and TRUMPF (**6**). These workers all agree that relatively low intensities of visible radiant energy in the range of 10 foot candles or less induce almost as complete morphological development of the stems and leaves of such shoots as high intensities of many thousands of foot candles. When low intensities are used, the leaves become green, expand into flat lamina, and the portions of the stem first laid down are greatly shortened as contrasted with plants grown in the complete absence of radiant energy where the leaves fail to develop chlorophyll, frequently do not expand, and the stem portions first laid down are relatively long. Many shoots, such as those of the red kidney bean, emerge from the germination medium with a well defined plumular hook, which does not completely disappear in the total absence of visible radiant energy. After the irradiation of the plants with low intensities of visible radiant energy, the plumular hook disappears.

TRUMPF came to the conclusion that chlorophyll synthesis was not directly connected with the mechanism involved in the disappearance of those characteristics associated with growth in the total absence of visible radiant energy. With short low intensity exposures he was able to secure leaf expansion, internodal shortening, and disappearance of the plumular hook without any apparent synthesis of chlorophyll. His results also indicated that it was the longer wavelengths of the visible spectrum that were primarily influential in causing leaf expansion, but stem elongation was not greatly affected by the red. The blue was more effective in inducing shortening of the stem. He used relatively wide bands of the red and blue regions which were balanced to equal readings with a selective radiometer.

This report concerns a preliminary investigation[3] on the influence of spectrally controlled radiant energy on the growth and development of red kidney bean seedlings and other physiologically young plants.

[1] Contributions from the Hull Botanical Laboratory 523.

[2] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

[3] A portion of the investigation was conducted at the Hull Botanical Laboratories, University of Chicago, during the winter of 1937–1938. The remainder of the investigation was conducted in the Department of Horticulture, Purdue University Agricultural Experiment Station, during the year of 1939–1940.

Procedure

The plants were grown in a series of six compartments 32 inches square by four feet high. The compartments were arranged in two units of three compartments per unit in a temperature controlled dark room having facilities for mounting the radiation equipment outside the room so that the lamps had a minimum influence on temperature. Above each compartment was built the radiation system which consisted of a tray of angle iron for the dyed gelatin secondary filters, over which was suspended a 25-inch square glass-bottomed filter cell, eight inches deep, containing distilled water. Where necessary to completely remove red radiant energy, an additional cell of glass, $1\frac{3}{16}$ inch deep by 24 inches square, containing a concentrated solution of copper sulphate, was immersed in the primary filter cell. This system eliminated the necessity of free surfaces of copper sulphate solutions above the plants. The lamp equipment was mounted above the filter cells, and consisted of 1500-watt vapor proof aluminum reflectors of the concentrating type. The details of the lamp equipment and filter systems on five of the six compartments are presented in table I.

TABLE I

DETAILS OF THE RADIATION SYSTEMS

WAVELENGTH LIMITS, Å	RADIATION SOURCE	PRIMARY AQUEOUS FILTER	SECONDARY FILTER DYED GELATIN FILM
BLUE 4047 and 4358 Hg lines	400-watt type H_1 high pressure Hg arc	10 cm. water and 3 cm. – 25 per cent. $CuSO_4 \cdot 5H_2O$	Victoria Pure Blue BO and Crystal Violet
YELLOW-GREEN 5461, 5770 and 5791 Hg lines	400-watt type H_1 high pressure Hg arc	10 cm. water and 3 cm. – 25 per cent. $CuSO_4 \cdot 5H_2O$	Pontamine Fast Yellow 5GL and Orange G
RED 6400 to approximately 9,000 continuous	300-watt gas-filled tungsten filament	10 cm. water	Chrysoidine Y and Crystal Violet—2 panes ⅛ inch Corning Aklo plate infrared absorbing glass
FAR RED 6800 to approximately 12,000 continuous	150-watt gas-filled tungsten filament	10 cm. water	Chrysoidine Y and Victoria Pure Blue BO
NEAR INFRARED 7200 to approximately 12,000 continuous	500-watt gas-filled tungsten filament	10 cm. water	Orange G, Pontamine Fast Red 8BL, and Acid Film Green 1854

The sixth compartment served as a dark plot. In some of the experiments, the near infrared treatment was eliminated and a second dark plot included. Figure 1 is a graph of the transmission spectra of the filter systems used.

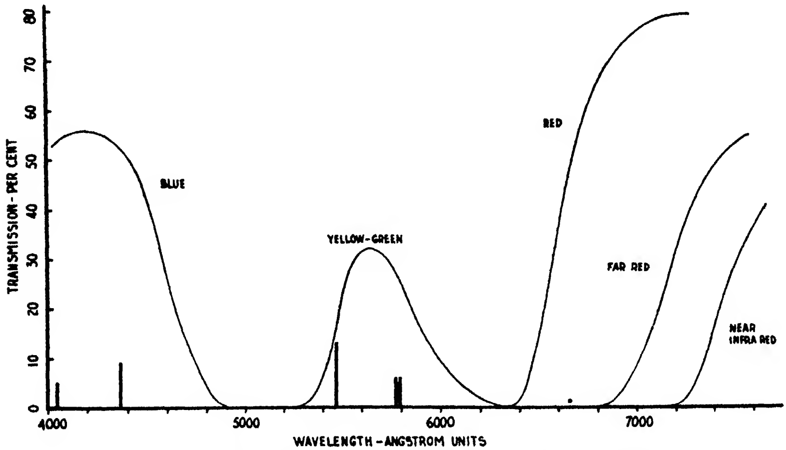


FIG. 1. Transmission spectra of the filter systems.

The energy was balanced with a thermopile which was mounted 20 cm. above the gravel surface and covered with a filter cell containing 18 mm. of ferrous ammonium sulphate saturated at 22° C., as described by PFUND (3). This filter efficiently absorbs radiant energy beyond about 8000 Å. with relatively little absorption within the range of the visible spectrum. The radiant energy for each plot was adjusted to the values presented in the tables of results. The final adjustments were obtained by placing narrow strips of black paper across the filter panes. It was assumed tentatively that the energy beyond 8000 Å. has little effect, this assumption being based upon a preliminary experiment in which no effects were obtained with radiant energy beyond this limit. For this preliminary experiment an irradiance of 1500 ergs per sq. cm. per second from an incandescent lamp source was filtered with 10 cm. of distilled water and a 3.5-mm. Corning no. 254 infra-red transmitting glass with transmission from 8000 Å. to 12000 Å. The results secured indicated that no appreciable effects on red kidney bean occur when the plants were irradiated with these wavelengths.

The plant material was grown in subirrigation gravel culture beds, 6 inches deep by 28 inches square. Tap water was supplied to the beds automatically every four hours by means of a time switch and a centrifugal pump. A pair of small fans circulated air up over the beds at a sufficiently rapid rate to maintain the temperature of the compartments the same as that of the room. The irradiation was applied continuously with no dark periods, except for the experiments reported in table VI, where the irradia-

tion was applied both continuously and for ten per cent. of the daily cycle, or 2.4 hours daily. Where high humidities were used, the air was kept supplied with water vapor from a small spray unit placed in front of a large fan. The temperature was maintained at 25° C.

Seeds of red kidney bean (*Phaseolus vulgaris*) were selected for uniform size. The variation in total seed weight per plot in any one experiment was not more than 0.5 per cent. There was considerable variation, however, in size of seed selected for the different experiments. The seeds were sown at a depth of one inch with a spacing of 2×2 inches. As soon as the plumular hook appeared above the gravel, the irradiation was applied. The plants were harvested 14 days from seeding.

Length measurements were made of the hypocotyl and first and second internodes. The internodes in this study were numbered from the cotyledon up the stem in the order of development. Fresh and dry weight were taken for the hypocotyl, first internode, cotyledons, first leaves and roots. Those portions of the plant above the first leaves were weighed as one fraction. The plant material was dried for 18 hours at 100° C. in a forced draft oven.

The leaf pigments were extracted with 100 ml. of acetone from 5 gm. of fresh leaf tissue. The relative chlorophyll concentration was then determined with a visual spectrophotometer from the specific transmissive index of the acetone solution using the 6630 Å. absorption band of chlorophyll.

Where transfers were made to the greenhouse from the radiation rooms, the greenhouse temperatures were maintained at approximately 19° C. during the day and 13° C. during the night. The short day condition was 9 hours long. Two long day conditions were used, one with 9 hours daylight supplemented with 15 hours of irradiation with 10 foot candles from an incandescent lamp or 9000 foot candle minutes nightly. The second long day treatment consisted of 9 hours daylight supplemented with equal energy of 9000 foot candle minutes supplied by intermittent irradiation with 200 foot candles on for 5 per cent. of the time or 1.5 minutes half-hourly. These greenhouse irradiation conditions were used because they were already in operation in connection with other experiments.

Seeds of pea (*Pisum sativum*), variety Little Marvel, were selected for uniform weight and sown with the same spacing and at the same depth as the red kidney bean, using the same wavebands and temperature. Length measurements were made of six internodes above the cotyledons and fresh and dry weights of leaves, stems, roots, and cotyledons were obtained. The plants were dried at 100° C. for 18 hours.

Pea, maize (*Zea mays*) and soybean (*Soya max*) seeds and small potato tubers (*Solanum tuberosum*) were selected for uniform size and these, together with tomato (*Lycopersicum esculentum*) and cocklebur (*Xanthium*

pennsylvanicum) seed, were grown under far red treatment (6800 Å. to 12000 Å.), and in complete darkness, all other experimental conditions being similar to those outlined above.

Results and discussion

Of the five bands of radiant energy used, the yellow-green and red caused the most pronounced morphological effect as contrasted with plants grown in the total absence of radiant energy. The far red band produced almost as great an effect on the development of the plants as the yellow-green and red, but the blue and near infrared bands had much less effect. On the basis of the data in the tables, the near infrared appears to be almost as effective as the blue, but it should be noted that the energy of the near infrared was 15 times that of the other plots.

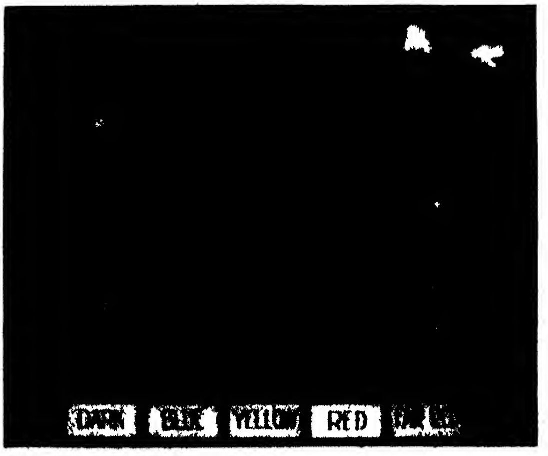
With the yellow-green and red bands of radiant energy, the hypocotyl and first internode lengths of red kidney bean were shortened (tables II, III, IV, and VI) and there was an increased development of the epicotyl above the first internode (plate I and fig. 2). Marked leaf expansion occurred under these wavelengths together with the rapid disappearance of the plumular hook.

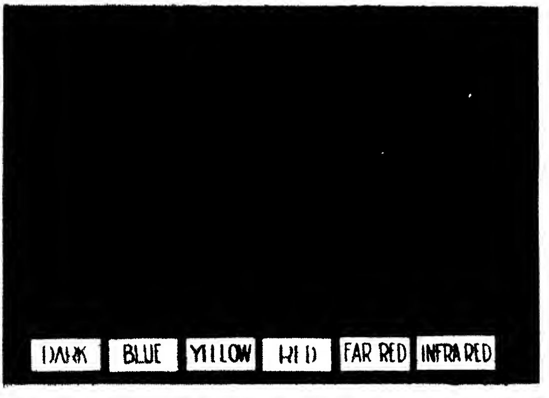
Under the blue, there was some shortening of the hypocotyl and first internode. The apical hook originating as a plumular hook, moved toward the tip of the plant and at the time of harvest had become a petiolar hook of the first leaves. Little leaf expansion occurred. Under the near infrared, the plumular hook disappeared entirely and some leaf expansion took place.

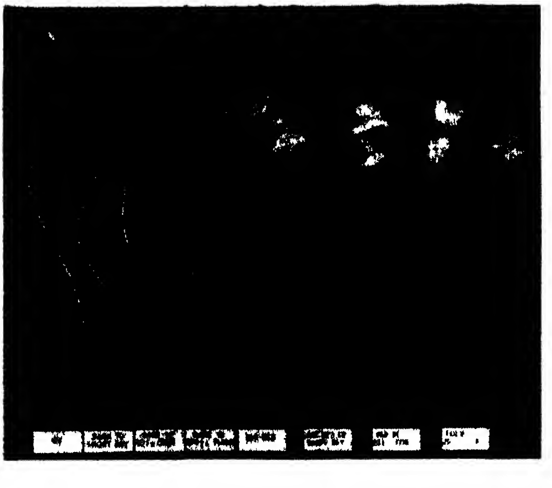
The dry weights show that all of the spectral regions, especially the red, accelerated the movement of the food reserves from the cotyledons into the extremities of the plant, the accelerated movement taking place both up the stem into the terminal portions of the shoot, and down into the root system. The dry weights of these parts increased considerably over similar fractions of plants grown in complete darkness or in the other spectral regions (fig. 3). In plants grown in the complete absence of radiant energy, the hypocotyl and first internode, both adjacent to the cotyledonary node, received the major portion of the food reserves translocated from the cotyledons.

The percentage of dry matter was at a maximum in the longer wavelength treatments. The top-root ratio was at a minimum under the same conditions as a result of the accelerated tendency of the food reserves to move into the root system.

This whole series of reactions which was brought about most effectively by the longer wavelengths of the visible spectrum does not appear to be directly related to respiration since, as shown in table II, there does not appear to be any significant difference in loss of dry matter by the plants


RED


BLUE
FAR RED
INFRA RED



as calculated from the difference between the total dry weight at harvest and the dry seed weight minus the seed coat.

Neither do these responses appear to be related to the amount of chlorophyll present in the leaf. Table II presents the relative concentration of chlorophyll per unit of fresh weight of leaf tissue. The highest chlorophyll

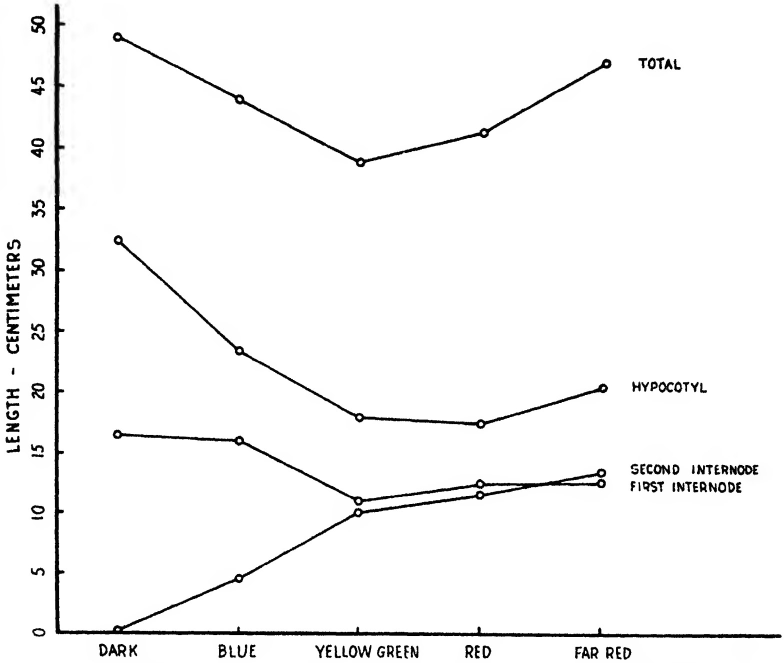


FIG. 2. Stem lengths of red kidney bean seedlings exposed to 500 ergs per sq. cm. · second of filtered radiant energy of the regions indicated.

PLATE III

EFFECT OF WAVELENGTH OF RADIATION ON THE GROWTH RESPONSE OF BEAN AND PEA

TOP.—Bean plants showed the maximum leaf expansion, development of the second internode, and the shortest hypocotyls in the yellow, red and far red. In the far red, only a trace of chlorophyll developed, whereas in the red, yellow and blue, the leaves were quite green. The development of the plants under the blue was similar to that of the plants grown in the dark with the exception of the presence of chlorophyll in those portions of the leaves exposed to the blue irradiation.

CENTER.—Pea, showing relationships similar to those exhibited by bean, although the differences occurring under the various spectral regions are not as marked.

BOTTOM.—Bean plants transferred from the dark and far red treatments to the greenhouse. The dark conditioned plants expanded their leaves and developed chlorophyll with a denser green color apparent in the long day treatment than in the short day condition. The plants conditioned by the far red failed to develop chlorophyll in the leaves previously exposed to the far red radiation. Those leaves in the bud at the time of transfer unfolded and developed chlorophyll.

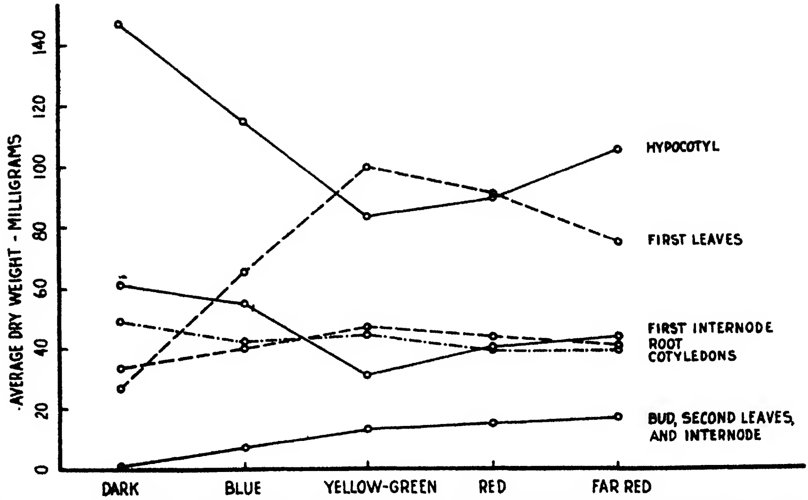


FIG. 3. Average dry weight per plant of various fractions of red kidney bean.

TABLE II

THE GROWTH RESPONSE OF RED KIDNEY BEAN SEEDLINGS* TO A CONTINUOUS IRRADIANCE OF 500 ERGS PER SQ. CM. PER SECOND AT 25° C.

PLANT FRACTION	DARK	BLUE	YELLOW-GREEN	RED	FAR RED
			LENGTH		
	cm.	*cm.*	*cm.*	*cm.*	*cm.*
Second internode	0.1	4.5	10.0	11.4	13.6
First internode	16.6	15.9	11.0	12.5	12.8
Hypocotyl	32.5	23.6	17.9	17.5	20.3
Total	49.2	44.0	38.9	41.4	46.7
			DRY WEIGHT		
	mg.	*mg.*	*mg.*	*mg.*	*mg.*
Bud, second leaves and internode	1	7	13	15	17
First leaves	27	65	99	90	74
First internode	61	55	31	40	43
Hypocotyl	147	114	83	89	104
Cotyledons	49	42	44	39	39
Root	34	40	46	43	40
Total	319	323	316	316	317
Percentage dry matter	4.9	5.6	6.0	6.0	5.9
Mg. respired	129	125	132	131	130
Top-root ratio	6.9	6.0	4.9	5.5	6.0
Relative chlorophyll concentration	0	14.5	27.0	16.5	0.9

* Plants per treatment: 100.

concentration appeared in the yellow-green. Since the leaves were not expanded in the blue, only a relatively small amount of surface was exposed to the radiant energy. In these exposed regions the green coloration appeared to be more intense than in any of the other plots, even though the extraction data, based on total leaf weight, does not show this.

The plants irradiated with the far red were consistently nearly as fully developed morphologically as those in the yellow-green and red plots, but the leaves contained only a trace of chlorophyll. SAYRE (5) has reported that chlorophyll is not formed to any appreciable extent by wavelengths longer than 6800 Å. The results of this experiment are in harmony with his conclusion. Chlorophyll synthesis therefore would not appear to be directly connected with the morphological responses obtained, since almost the maximum response occurred in the far red where almost no chlorophyll was present and a minimum response in the blue where a relatively high chlorophyll concentration obtained. These conclusions are in accord with those of TRUMPF who obtained similar morphological development without the synthesis of chlorophyll when the seedlings were treated with very short exposures to white irradiation.

TABLE III

THE GROWTH RESPONSE OF RED KIDNEY BEAN SEEDLINGS* TO A CONTINUOUS IRRADIANCE OF 100 ERGS PER SQ. CM. PER SECOND AT 25° C. AND 25 PER CENT. RELATIVE HUMIDITY

PLANT FRACTION	DARK	BLUE	YELLOW-GREEN	RED	FAR RED	INFRA-RED†
			LENGTH			
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
Second internode	0.4	4.1	9.3	9.5	8.9	5.8
First internode	21.5	15.3	15.2	14.4	15.6	18.3
Hypocotyl	23.6	20.6	15.4	15.0	16.1	21.6
Total	45.5	40.0	39.9	38.9	40.6	45.7
			DRY WEIGHT			
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Bud, second leaves and internode	1	6	11	13	11	9
First leaves	20	36	43	45	44	41
First internode	63	53	45	43	49	58
Hypocotyl	91	92	73	73	81	90
Cotyledons	70	54	62	56	60	54
Root	28	28	33	32	37	29
Total	273	269	267	262	282	281
Percentage dry matter	5.2	5.9	6.4	6.1	6.5	5.9
Top-root ratio	6.2	6.7	5.2	5.5	5.0	6.8

* Plants per treatment: 40.
† Adjusted to 1500 ergs per sq. cm. per second.

Several minor experiments were conducted on the plants irradiated with wavelengths longer than 6800 Å. as obtained in the far red treatment. Since the expanded leaves of these plants contained very little chlorophyll, and were yellowish in color, they were transferred to the greenhouse to full sunlight nine hours daily in order to determine whether chlorophyll would be synthesized normally in these plants. It was found that fully expanded leaves previously irradiated with the far red did not synthesize any chlorophyll in seven days time (plate I). At the end of a week the leaves were still alive but completely lacking in green coloration. The young leaves which unfolded after the transfer to the greenhouse developed chlorophyll quite normally. Plants taken from the dark to the greenhouse expanded their leaves which became green. In this case, the color was more intense in the long days than in the short days, as indicated in the photograph. Some of the plants from the far red were placed in tap water and some in a complete nutrient solution, but the effect was the same in both cases.

TRUMPF likewise obtained somewhat similar results with his plants which developed expanded leaves containing no chlorophyll under the influence of short exposures to white irradiation. His plants failed to develop chlorophyll in the older leaves on transfer to the greenhouse.

TABLE IV

THE GROWTH RESPONSE OF RED KIDNEY BEAN SEEDLINGS* TO A CONTINUOUS IRRADIANCE OF 100 ERGS PER SQ. CM. PER SECOND AT 25° C. AND 75 PER CENT. RELATIVE HUMIDITY

PLANT FRACTION	DARK	BLUE	YELLOW-GREEN	RED	FAR RED	INFRA-RED†
			LENGTH			
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
Second internode	2.3	5.5	11.4	11.7	9.9	5.5
First internode	23.8	16.5	16.4	14.6	15.5	18.2
Hypocotyl	21.6	18.5	14.6	15.4	16.2	20.7
Total	47.7	40.5	42.4	41.7	41.6	44.4
			DRY WEIGHT			
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Bud, second leaves and internode	2	7	16	15	12	7
First leaves	24	45	56	55	50	42
First internode	64	51	44	37	43	52
Hypocotyl	81	79	74	73	75	83
Cotyledons	46	44	40	42	45	46
Root	31	35	42	45	41	38
Total	248	261	272	267	266	268
Percentage dry matter	4.8	5.3	5.6	5.7	5.8	5.4
Top-root ratio	5.5	5.2	4.5	4.0	4.4	4.8

* Plants per treatment: 25.
† Adjusted to 1500 ergs per sq. cm. per second.

TABLE V

THE GROWTH RESPONSE OF PEA, VARITY LITTLE MARVEL, SEEDLINGS* TO A CONTINUOUS IRRADIANCE OF 100 ERGS PER SQ. CM. PER SECOND AT 25° C. AND 75 PER CENT. RELATIVE HUMIDITY

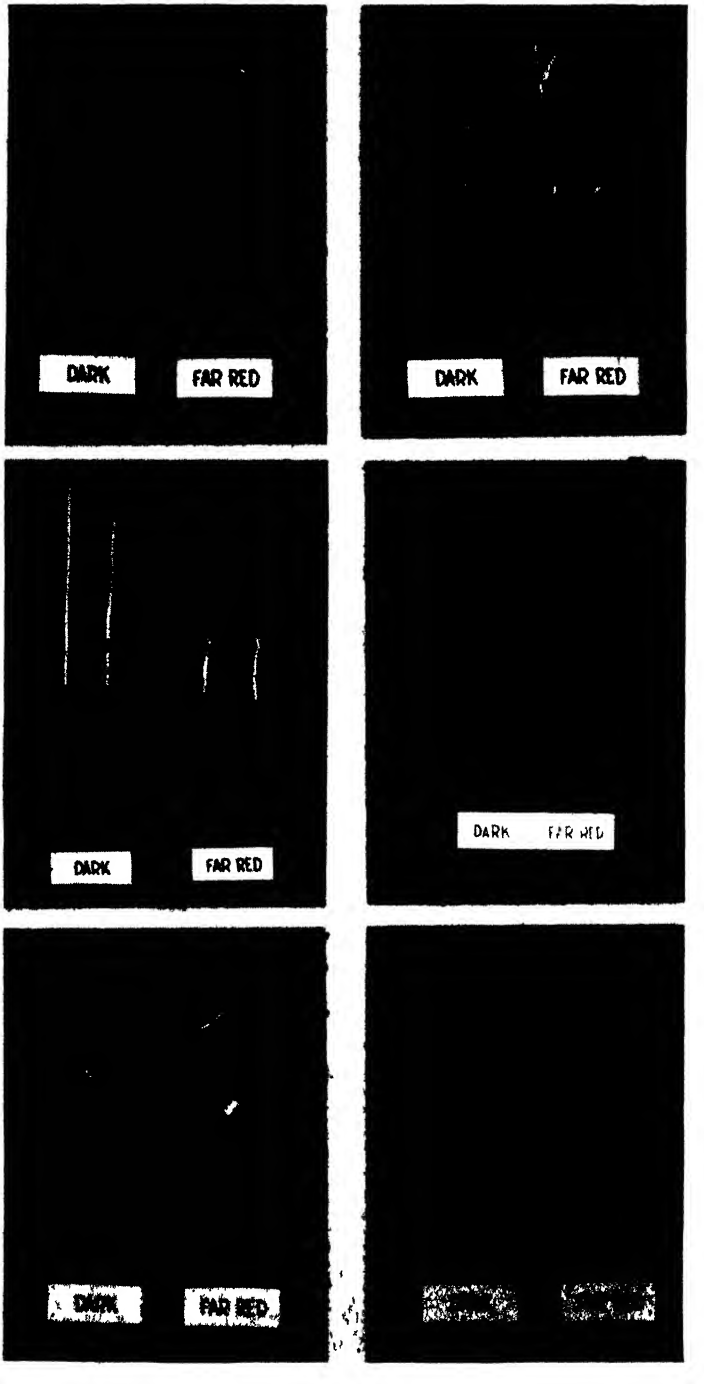
PLANT FRACTION	DARK	BLUE	YELLOW-GREEN	RED	FAR RED	INFRA-RED†
			LENGTH			
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
Internode VI	0.4	0.8	0.9	0.6	1.1	1.1
Internode V	6.7	3.5	2.8	2.3	2.9	3.9
Internode IV	12.2	4.6	3.4	2.9	3.8	6.0
Internode III	9.9	6.0	3.2	3.6	4.1	6.1
Internode II	2.5	3.1	2.0	2.5	2.5	3.0
Internode I	3.9	3.8	3.1	3.5	3.7	3.7
Total	35.6	21.8	15.4	15.4	18.1	23.8
			DRY WEIGHT			
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Leaves	9	22	28	26	27	21
Stems	63	41	32	30	37	41
Cotyledons	46	44	44	37	43	41
Roots	24	24	27	25	27	24
Total	142	131	131	118	134	127
Percentage dry matter	5.1	5.4	6.0	5.7	5.7	5.1
Top root ratio	3.0	2.6	2.3	2.3	2.4	2.6

* Plants per treatment: 30.
† Adjusted to 1500 ergs per sq. cm. per second.

Data given in table II were secured at 500 ergs per sq. cm. per second and in table III at 100 ergs per sq. cm. per second. These show the same general trend in all cases. It was not practical to reduce the intensity much further than 100 ergs and therefore intermittent irradiation was used with the lamps on for 2.4 hours daily so as to reduce the total daily radiant energy to 10 per cent. of the continuous irradiation. Height results for bean and pea under these conditions are given in table VI. It can be seen here that the differences were considerably less marked with intermittent irradiation, but there was still a great deal of influence on the development of the plants.

Tables III and IV give comparative data for results secured at 25 per cent. and 75 per cent. relative humidity. The same trend of morphological development was observed in both cases although a slightly higher percentage of dry matter occurred at the lower humidity, and the leaf expansion was not as great. The actual weight and height figures cannot be directly compared, however, since the initial seed weights were not the same.

Several experiments were run with the Little Marvel variety of pea and a set of typical results are given in table V. The weight results were similar to those obtained with bean. The leaves were somewhat more fully ex-


DARK
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panded under the longer wavelengths and the total height was less. Internode lengths show that as the internodes become further removed from the cotyledons, they become shorter under all wavelengths of radiant energy, with the greatest shortening with the yellow-green, red, and far red (plate III). The leaves were heavier under the longer wavelengths, and the dry weights of the stems less.

Since bean plants develop with expanded leaves under radiant energy of wavelengths longer than 6800 Å. without appreciable development of chlorophyll, a number of other species of plants was grown without radiant energy and with this region of the spectrum applied continuously at 2000 ergs per sq. cm. per second. As can be seen from the photographs presented in plate II, the responses of all dicotyledonous plants were similar to those of the bean, with increased leaf expansion, shortened hypocotyl, and the disappearance of the plumular hook where either was present. In the case of maize, leaf expansion was greatly increased under the far red. Only a trace of green coloration could be observed in any of these plants.

Red kidney bean plants were grown in a preliminary experiment using a complete nutrient solution containing both nitrate and ammonium nitrogen. The same type of results was secured with a complete nutrient solution as with tap water.

Summary and conclusions

Seedlings of red kidney bean (*Phaseolus vulgaris*) and pea (*Pisum sativum*) were grown in gravel culture, subirrigated with tap water, in the complete absence of visible and near visible radiant energy, and irradiated with 500 and 100 ergs per sq. cm. per second of five bands of visible and near infrared radiation including: blue (4047, and 4358 Å. Hg lines); yellow-green (5461, 5770, and 5791 Å. Hg lines); red (6400 to approximately 9000 Å. incandescent); far red (6800 Å. to approximately 12000 Å incandescent); and 1500 ergs per sq. cm. per second of near infrared (7200 Å to approximately 12000 Å incandescent).

Seedlings of pea, maize, soybean, tomato, and cocklebur, and sprouts of

PLATE IV

FAR RED RADIATION

Influence of low intensity far red radiation (6800–12000 Å.) on the morphological development of pea (top left), maize (top right), potato (center left), tomato (center right), soybean (bottom left), and cockle bur (bottom right). The far red caused the disappearance of the plumular hook in the dicotyledonous plants, a shortening of the hypocotyl where it was present, and increased the leaf expansion and development of the apical bud. Only a trace of chlorophyll occurred in the plants treated with the far red radiation.

TABLE VI

THE HEIGHT RESPONSE OF RED KIDNEY BEAN AND PEA, VARIETY LITTLE MARVEL, SEEDLINGS TO RADIANT ENERGY APPLIED CONTINUOUSLY AND FOR 10 PER CENT. OF THE DAILY CYCLE AT 25° C.

PLANT	IRRADIATION TREATMENT	PLANT FRACTION	DARK	BLUE	YELLOW-GREEN	RED	FAR RED	INFRA-RED*
Red kidney bean	Continuous	Second internode	2.3	5.5	11.4	11.7	9.9	5.5
		First internode	23.8	16.5	16.4	14.6	15.5	18.2
		Hypocotyl	21.6	18.5	14.6	15.4	16.2	20.7
		Total	47.7	40.5	42.4	41.7	41.6	44.4
	10 per cent. daily cycle†	Second internode	1.5	4.4	8.0	10.9	4.9	3.1
		First internode	23.2	21.7	21.2	17.4	21.0	22.5
		Hypocotyl	23.0	20.2	17.3	19.7	19.8	22.5
		Total	47.7	46.3	46.5	48.0	45.7	48.1
Pea	Continuous	Total	35.6	21.8	15.4	15.4	18.1	23.8
	10 per cent. daily cycle†	Total	35.6	27.3	24.0	23.4	28.2	33.2

* Adjusted to 1500 ergs per sq. cm. per second.

† All heights multiplied by factor 1.07 for bean, and 1.10 for pea, in order to adjust the total height of the dark plants to the same value for continuous irradiation and for irradiation applied for 10 per cent. of the daily cycle.

potato were grown in the absence of radiant energy and irradiated with 2000 ergs per sq. cm. per second of far red irradiation.

With red kidney bean, the yellow-green and red bands caused the most pronounced morphological differences as compared with plants grown in the complete absence of radiant energy. The far red produced almost as great an effect, but the blue and high intensity infrared were much less effective. The longer wavelengths of the visible spectrum caused a marked increase in leaf size and expansion and a shortening of the hypocotyl and first internode. The dry weights of the hypocotyl and first internode were greatly reduced, whereas the dry weights of the root, first leaves and the epicotyl above the first leaves were materially greater than those of the dark conditioned plants.

The data presented indicate that in the absence of radiant energy the major portion of the reserve material translocated from the cotyledons remained in those parts of the plant immediately adjacent to the cotyledonary node, *i.e.*, the hypocotyl and the first internode. The longer wavelengths of the visible spectrum increased the total amount of reserves translocated from the cotyledons, and also greatly increased the proportion translocated beyond the hypocotyl and first internode, that is, to the epicotyl above the first internode, and to the roots. The accelerated movement was considerably greater toward the apical portions of the stem than to the roots.

It is concluded that the process is not directly related to chlorophyll synthesis, since a minimum morphological response was produced by the blue region with a strong development of chlorophyll, and almost a maximum morphological response occurred in the far red where only a trace of chlorophyll appeared.

Pea gave much the same type of response as red kidney bean as to total stem length, leaf expansion and weight and translocation of material from the cotyledons.

The far red had essentially the same type of effect on maize, soybean, tomato, cocklebur, and potato as it had on red kidney bean. The far red region caused a disappearance of the plumular hook of the dicotyledonous plants, a shortening of the hypocotyl where present, an increased leaf expansion and an increased size of roots. Only a trace of green color was apparent in the leaves of any of these plants under the far red.

The author wishes to express his sincere appreciation to Dr. C. A. Shull for his many helpful suggestions during this investigation.

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ABSORPTION AND UTILIZATION OF RADIOACTIVE CARBON DIOXIDE BY SUNFLOWER LEAVES

JAMES H. C. SMITH AND DEAN B. COWIE[1]

(WITH TWO FIGURES)

Introduction

The absorption of carbon dioxide by leaves is one of the important steps in the over-all process of photosynthesis. Leaves, although unilluminated, absorb carbon dioxide reversibly from the surrounding atmosphere. Some leaves also contain considerable quantities of carbon dioxide combined as carbonates and bicarbonates. Both of these properties are especially marked in sunflower leaves.

Analysis of carbon dioxide absorption by sunflower leaves has indicated that absorption takes place in at least three different ways: by physical solution in the water of the sap; by chemical reaction with buffer substances dissolved in the sap; and by insoluble alkaline earth carbonates (7). To what extent each of these reactions participates in the total absorption by the living leaf and to what extent the absorption of carbon dioxide in the absence of light concerns the photosynthetic process were not ascertainable. When radioactive carbon became accessible a means was provided for investigating these relations, and it is the purpose of this paper to report some of the results of such an investigation.

Methods and results

PREPARATION AND MEASUREMENT OF RADIOACTIVE CARBON DIOXIDE

The radioactive carbon was made by bombarding boron oxide with 2 MEV deuterons in the high-voltage equipment of the Department of Terrestrial Magnetism of the Carnegie Institution of Washington (8). The oxides of carbon were removed from the boron oxide glass by heating and were converted completely into carbon dioxide by combustion over copper oxide (9). Ordinary carbon dioxide was used as carrier for the radioactive gas. The radioactive carbon dioxide was purified by solution in potassium hydroxide followed by liberation with lactic acid. Extraneous gases were removed from the alkaline solution by evacuation before addition of the acid. In this way samples of pure carbon dioxide were obtained.

In this paper the term "radioactive carbon dioxide" is used to denote carbon dioxide to which a sample of radioactive carbon dioxide has been added for indicator purposes. The radioactivity of the resulting mixture

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enables the reactions of this particular sample of gas to be followed quantitatively. The term radioactive is preferred to the terms "labeled" or "tagged," since the two latter terms are not specific and apply to samples containing mass isotopic indicators as well as radioactive isotopic indicators.

For determination of the radioactivity of the carbon dioxide, a measured quantity of the gas was drawn into an evacuated flask containing 5.00 ml. of N potassium hydroxide. The alkaline solution containing the radioactive carbonate (4.00 ml.) was pipetted into a hard rubber cup, 4.5 cm. in diameter and 0.5 cm. deep. After being covered with a 0.1-mm. aluminum foil, the cup was placed in position under an ionization chamber attached to a LUTZ-EDELMANN electrometer (1) by means of which the radioactivity was measured.

In order to increase the reproducibility of the determinations of radioactivity, the inside of the cup was paraffined to prevent creeping of the solution and the surface of the alkaline solution was touched with a wire moistened with olive oil to eliminate certain objectionable surface-tension effects.

The radioactivities of the various fractions of carbon dioxide obtained during the course of an experiment were compared by referring them to a common time by means of the half-life constant, 20.35 minutes. This half-life constant was determined by a series of separate experiments under the conditions just described and is probably accurate to within ± 0.23 minutes.

CARBON DIOXIDE EXCHANGE IN SUNFLOWER LEAVES IN THE DARK

From the previous work (7) it appears that sunflower leaves contain a considerable quantity of carbon dioxide combined in the form of salts. These may constitute a reservoir of carbon dioxide potentially available for photosynthesis. In order to determine whether the carbon dioxide absorbed from the surrounding atmosphere becomes a part of this reservoir the following experiment was performed.

Sunflower leaves (10.00 gm.) were cut into pieces with an area of about one square inch. These pieces were put into a reaction flask attached to a manometer-pump system similar to that previously described (7). The flask was immersed in a constant-temperature water bath maintained at 15.0° ± 0.1° C. After the flask containing the leaves had been exhausted by means of a SPRENGEL pump, a known volume of radioactive carbon dioxide was admitted to the flask. (The apparatus used in these experiments differed from the one previously described (7) in that the gas introduced into the reaction vessel was measured volumetrically rather than manometrically.) By comparing the change in pressure produced with the change caused by introduction of the same volume of an insoluble gas (nitrogen) the amount of carbon dioxide absorbed by the leaves was calcu-

lated (table II). The amount of carbon dioxide which reacted chemically (table II) was estimated by subtracting the amount physically dissolved in the sap of the leaves from the total amount absorbed. The amount of carbon dioxide physically dissolved by the sap of the sunflower leaves was calculated from the water content of the leaves, the partial pressure of the carbon dioxide in the flask at equilibrium, and the solubility of the carbon dioxide in the sap, *viz.*, 0.926 ml. per gm. of water, per atmosphere of carbon dioxide pressure, volume reduced to standard conditions (7).

The carbon dioxide pumped from the reaction flask by means of the SPRENGEL pump and collected in another gas buret (table I) accounted for all of the carbon dioxide admitted initially to the reaction flask. The radioactive carbon dioxide recovered by pumping was less than the amount introduced. In all cases the amount retained is a larger proportion of the amount introduced than the proportion retained by an equal weight of water. These results indicate that an exchange of carbon dioxide takes place within the leaves.

By acidification of the leaves, it was shown that some of the radioactive carbon dioxide absorbed from the atmosphere had replaced carbon dioxide in carbonates and bicarbonates. After the absorption experiments had been completed, and as much as possible of the carbon dioxide had been removed by evacuation, the leaves were treated with 10 ml. of 6 N hydrochloric acid.

TABLE I

RETENTION OF CARBON DIOXIDE BY SUNFLOWER LEAVES AND BY WATER

MATERIAL TESTED	PRESSURE CO_2	VOLUME INTRODUCED	VOLUME REMOVED	RADIOACTIVITY INTRODUCED	RADIOACTIVITY REMOVED	PERCENTAGE RETAINED
	atm.	*ml.*	*ml.*	*units*†	*units*†	
Sunflower leaves, 10 grams fresh weight	0.2620*	13.51	13.85	2429	2289	5.76
	0.2645*	14.08	14.73	1132	1042	7.95
	0.2870	14.72	15.13	748	728	2.76
	0.5608	27.79	28.28	876	790	9.75
	0.6124*	30.96	31.57	7824	6691	14.48
	0.6520*	32.16	32.72	5427	5267	2.95
	0.7139	35.34	35.55	1155	1074	7.01
Water, 10 ml.	0.2351	11.12	11.17	1511	1484	1.82
	0.4686	22.15	22.34	6322	6620	−4.72

* The measuring cell in these experiments was covered with cellophane.

† The units of radioactivity are entirely arbitrary and have no absolute significance. The units used in any one experiment are consistent and comparable.

The carbon dioxide liberated (table II) was pumped from the reaction flask and its radioactivity determined. This gas contained considerable quantities of radioactive carbon dioxide (table II) which must have been retained by the leaves in the form of carbonates and bicarbonates.

Separate tests showed that 10.00 ml. of water when placed in the reaction vessel retained only a small quantity of the radioactivity introduced into the reaction system. The test was made by pumping from the water as much as possible of the radioactive carbon dioxide, adding sodium carbonate to the water, and liberating the carbon dioxide with hydrochloric acid. This carbon dioxide was recovered by pumping, and in two experiments was shown to contain 1.47 and 0.21 per cent. of the activity initially introduced, corresponding to 0.16 and 0.05 ml., respectively.

Still further evidence for the exchange of carbon dioxide within the leaf was the observation that leaves which had been saturated with radioactive carbon dioxide retained their radioactivity after evacuation and after standing in the dark for as long as two hours.

One sample of leaf discs which had been saturated with radioactive carbon dioxide contained 82 units of radioactivity. After evacuation and flushing five times with ordinary carbon dioxide, this sample still contained 23 units of radioactivity, or 27 per cent. of the amount initially absorbed. This approximates the amount retained in the absorption experiments the results of which are recorded in table II, *i.e.*, 22.7 per cent. (average value).

Another leaf sample, saturated with radioactive carbon dioxide and stored in the dark for 129 minutes, possessed the following activities: initial activity, 1195 units; activity after storage, 212 units; activity after acidification, 9 units. That most of this activity was retained in the leaf as carbonate or bicarbonate is shown by the large loss of activity on treatment with hydrochloric acid.

From the results reported in table II it is apparent that the amount of carbon dioxide retained closely parallels the amount of carbon dioxide that had reacted chemically in the leaf. If a reasonable explanation of the mechanism of the exchange can be obtained it may provide insight into the carbon-dioxide-absorption process, for these two processes are undoubtedly closely allied.

A reasonable conception of the absorption and exchange processes has been obtained from the experiments on the reversible absorption of radioactive carbon dioxide. According to this conception, of the total carbon dioxide absorbed (a), a portion (r), combines chemically with certain constituents within the leaf. Part of this combines with buffer substances dissolved in the sap of the leaf, and another part (fr) reacts with insoluble carbonates. The latter is thus dissolved in the leaf sap. There is already present in the leaf sap a residual amount of bicarbonate (b), with which the absorbed carbon dioxide and bicarbonate ion formed by dissolution of the insoluble carbonates, are pooled. The amount of exchange (e) observed is the ratio of the amount of carbon dioxide retained on evacuation to the total amount in the pool at equilibrium multiplied by the total amount absorbed.

TABLE II

THE EXCHANGE OF CARBON DIOXIDE IN SUNFLOWER LEAVES

PRESSURE CO_2	VOLUME OF CARBON DIOXIDE				EXCHANGE CALCULATED
	ABSORBED	REACTED	RETAINED†	LIBERATED	
atm.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
0.262*	3.46	1.50	0.95		0.92
0.265*	3.95	1.81	0.69	19.2	1.07
0.287	3.73	1.59	0.94	28.3	0.97
0.561	6.37	2.23	1.54	28.3	1.38
0.612*	7.51	3.10	1.76	33.2	1.80
0.652*	7.37	2.53	1.48	19.3	1.55
0.714	8.15	2.87	1.72	32.4	1.74

* Measurements of radioactivity made with cup covered with cellophane window. In others an aluminum window was used.

† The amount retained was calculated from the amount of radioactivity liberated with acid after removing as much as possible of the carbon dioxide by pumping.

The algebraic equation representing this conception of the exchange process is

$$\text{(A)} \qquad \frac{b + fr}{b + fr + a}\, a = e$$

The amount of exchange calculated by this expression agrees well with the amount of exchange observed (table II). In the algebraic equation the values assigned to b and f are 0.20 and 0.70 ml., respectively. The values of a and r are taken from table II.

While direct evidence concerning the chemical nature of the insoluble substance in the living leaf which reacts with carbon dioxide (represented by the term fr) is not available, its chemical nature may be deduced indirectly. Previous work on killed sunflower leaves indicated that this insoluble carbonate was calcium carbonate (**7**). If this is the case, the experimental results obtained from the exchange reactions when substituted into the expression for the solubility product of calcium carbonate should yield a value for this constant which is in reasonable agreement with the values already reported. Inasmuch as the values obtained (table III) agree with the solubility product of calcium carbonate it may be assumed that the active substance is calcium carbonate.

The expression for the solubility constant of calcium carbonate is

$$\text{(B)} \qquad \frac{(Ca^{\cdot\cdot}) \cdot (HCO_3^-)^2 \cdot K''}{(H_2CO_3) \cdot K'} = K_{sp}$$

where the quantities in parentheses represent the activities of the components; K''/K' is the ratio of the secondary to the primary ionization constant of carbonic acid; and K_{sp} is the solubility product of calcium carbonate (**3**).

Assume that the concentration of the calcium ion is equal to the sum of the value *fr*, converted to moles per liter, plus the residual calcium ion present in sunflower-leaf sap (approx. 0.015 moles per liter). The total concentration of the calcium ion is shown in table III.

The bicarbonate ion was assumed to be equal to the sum of: the residual bicarbonate ion remaining fixed in the sap of the leaves (term *b*, equation A); the amount of carbon dioxide which reacted chemically, designated *c* (table II); and the amount brought into solution by dissolution of the insoluble calcium carbonate, *fr*. Algebraically this sum is $b + c + fr$, the values of which are given in table III.

The concentration of the dissolved carbon dioxide was calculated from the partial pressure of the carbon dioxide and its solubility in water at 15°, 0.0454 mol./kg./atm. (2).

At the ionic strength of sunflower-leaf sap, 0.2434 (7) the activity coefficient of calcium bicarbonate is 0.49 (3). The ratio K''/K' is 1/9100 (3).

By substitution of these values into equation B the following expression is obtained

$$\text{(C)} \qquad \frac{[0.49\,(fr + 0.015)]\,[0.49\,(b + c + fr)]^2}{9100\,(0.0454)\,P_{CO_2}} = K_{sp}$$

from which the values of K_{sp} reported in table III, were calculated. The

TABLE III

CALCULATION OF THE SOLUBILITY PRODUCT OF THE INSOLUBLE METAL CARBONATE, PRESUMABLY CALCIUM CARBONATE, FROM THE OBSERVATIONS ON ABSORPTION AND EXCHANGE OF CARBON DIOXIDE IN SUNFLOWER LEAVES

PRESSURE CO_2	$fr.$ + CA	BICARBONATE	CARBONIC ACID	K_{sp}
atm.	*M/L*	*M/L*	*M/L*	$\times 10^9$
0.262	0.02124	0.01578	0.01189	5.7
0.265	0.01858	0.01488	0.01203	4.4
0.287	0.02077	0.01582	0.01303	5.2
0.561	0.02529	0.02392	0.02547	7.3
0.612	0.02679	0.03032	0.02778	11.5
0.652	0.02429	0.02462	0.02960	6.4
0.714	0.02612	0.02836	0.03242	8.4
			Average	7.0

average value obtained for the solubility product is 7.0×10^{-9} at 15.0° C. This is in good agreement with the value obtained by FREAR and JOHNSTON which, extrapolated to 15° C., is 7.14×10^{-9}. From the agreement between the solubility product calculated from the data obtained from sunflower leaves and the solubility product found from physico-chemical measurement, it is apparent that calcium carbonate participates to an appreciable extent in the absorption of carbon dioxide by living sunflower leaves.

Additional evidence that the exchange in sunflower leaves may be almost completely accounted for by reaction of carbon dioxide with calcium carbonate is obtained from experiments in which leaf discs were saturated with radioactive carbon dioxide at about 5° C. and atmospheric pressure and then stored in air in the dark until all the free carbon dioxide had diffused out of the leaf. Referred to 10 gm. of leaf material, the amount of radioactive carbon dioxide retained, 2.49 ml., agreed well with the amount calculated from the solubility of calcium carbonate under the conditions obtained in the leaf, *viz.*, 2.65 ml. The amount of calcium carbonate potentially involved in the dissolution and exchange process corresponds to about 10.8 ml. of carbon dioxide. This is somewhat less than the total amount of carbon dioxide obtained from 10 gm. of sunflower leaves on acidification (table II) but this discrepancy may be due to incomplete mixing of the precipitated calcium carbonate with all of the calcium carbonate contained in the leaf.

By substitution of experimentally determined quantities in equation A, it is possible to calculate the amount of carbon dioxide absorbed by calcium carbonate in the leaf, f_r. Subtraction of this quantity from the total amount of carbon dioxide absorbed chemically gives an estimate of the amount of carbon dioxide absorbed by buffer substances. The values so calculated were found to be:

P_{CO_2}	0.262	0.265	0.287	0.561	0.612	0.652	0.714	atm.
Buffer abs.	0.39	1.17	0.56	0.40	1.00	0.88	0.89	ml.

In general the amount of carbon dioxide absorbed by buffers increased with increase in the partial pressure of the carbon dioxide. So far the nature of the buffer system has not been determined completely.

The pH of sunflower-leaf sap

From the data at hand it is possible to estimate the pH of the sap within the sunflower leaf. The ionization constant of carbonic acid has been reported in a previous paper, where the expression relating it to the pH of sunflower-leaf sap, expressed from the leaf, has been given. The equation was found to be:

(D) $$pH = 6.425 + 1.343 - 0.177 - pHCO_3 + pP_{CO_2}$$

(1.343, −logarithm of the solubility of carbon dioxide; 0.177, −logarithm of the activity coefficient of bicarbonate ion; and 6. 425, −logarithm of the ionization constant of carbonic acid). As is shown in table III, the total bicarbonate ion concentration in the sunflower leaf is known at various partial pressures of carbon dioxide. By substituting the appropriate functions of the bicarbonate ion concentrations and of the partial pressures of the carbon dioxide in equation D the values of the pH of the sunflower-leaf sap have been calculated.

P_{CO_2}	0.262	0.265	0.287	0.561	0.612	0.652	0.714
pH calc.	6.37	6.34	6.33	6.22	6.29	6.17	6.19
pH sap.	6.35	6.34	6.32	6.10	6.07	6.05	6.02

Comparison of these values with the values obtained by direct measurement of expressed sap, obtained in the previous investigation (7) and interpolated to the same partial pressure of carbon dioxide, shows that reasonable agreement exists between the two results, and that the intact leaf is a better buffer than the expressed sap.

Assimilation of carbon dioxide by unilluminated leaves

Unilluminated leaves combine chemically with carbon dioxide. This has been demonstrated by observing a "dark pick-up" of carbon dioxide immediately following photosynthesis by wheat leaves (4) and also by observing the carbon dioxide absorption by leaves from a number of species of plants under equilibrium conditions of carbon dioxide pressure (7). In sunflower leaves this chemical absorption is accomplished largely by reaction with buffers and insoluble carbonates, presumably calcium carbonate. Besides this, however, another type of absorption takes place in the dark in which carbon dioxide becomes bound much more firmly than in carbonates. Our experiments, as well as the earlier ones of Ruben, Kamen, and their associates (5, 6) have demonstrated this by the use of radioactive carbon dioxide. This absorption has been attributed to the formation of organic compounds which supposedly contain the radioactive carbon in carboxyl groups (6). Our experiments have given no definite knowledge concerning the nature of the compound formed by this reaction.

The leaf discs (4.5 cm. diameter) to be treated with radioactive carbon dioxide were placed in a test tube, evacuated with a Hyvac pump, and saturated with radioactive carbon dioxide at atmospheric pressure and at about 5° C. The leaf discs were freed from all carbonates by acidification with 2 ml. of 6 N hydrochloric acid and evacuation with an aspirator pump, warming the mixture in a water bath during the evacuation. All manipulations previous to the acidification were carried out in the dark.

The radioactivity of the leaf residue and acid solution was determined. Any activity observed was ascribed to noncarbonate, radioactive carbon.

The amount of noncarbonate, radioactive material usually formed in 10 gm. of fresh sunflower leaves by one treatment with radioactive carbon dioxide corresponded to absorption of approximately 0.304 ml. of carbon dioxide. The product formed is completely soluble in water. The radioactive carbon is bound so that it cannot be freed by hydrolyzing the material for a few minutes with either warm acid or alkali.

Only traces of this material were formed by leaves killed by freezing previous to the absorption of radioactive carbon dioxide, although freezing

subsequent to absorption did not destroy the material formed. It is probable that killing destroys the ability of leaves to form this material.

The amount of radioactive, noncarbonate material does not increase with time of contact between leaves and radioactive carbon dioxide, at least after fifteen minutes, as the following experiment shows: Four samples of sunflower leaves were kept in an atmosphere of radioactive carbon dioxide for varying lengths of time. These samples were removed from the radioactive gas at the time intervals indicated and were tested for radioactivity after acidification. From the data obtained it is apparent that no significant increase in the amount of noncarbonate compounds was observed after the first fifteen minutes. How short a time is necessary to reach the maximum concentration has not yet been determined.

Interval of storage (minutes)	15	29	44	59
Logarithm of activity referred	1.70	1.76	1.71	1.74
to common time	1.63	1.76	1.68	1.76

In a single experiment when the leaf was saturated with ordinary carbon dioxide before evacuation and exposure to an atmosphere in radioactive carbon dioxide, less than the average amount of radioactive carbon dioxide was fixed: average amount fixed, including several determinations not listed in table IV, 0.0393 ml.; amount fixed after pre-treatment with ordinary carbon dioxide, 0.0198 ml. (table IV).

There is indication that in the living leaf exchange takes place between ordinary carbon dioxide and the noncarbonate radioactive carbon fixed in the dark. In order to prove this conclusively, however, more critical experiments need to be performed (table IV, note e).

Multiple saturation with radioactive carbon dioxide increased the amount of noncarbonate radioactive compound. In one experiment when three discs of leaves (approx. 1.29 gm.) were evacuated and saturated three times with radioactive carbon dioxide; 0.0841 ml. of carbon dioxide was fixed as noncarbonate carbon whereas the average amount fixed by a single saturation was 0.0393 ml. (table IV).

Three types of exchange reaction suggest themselves: (1) a statistical exchange with a compound completely dissolved in the sap of the leaf; (2) an exchange depending on the dissolution of some material by increased partial pressures of carbon dioxide; and (3) an exchange brought about by alternate dissociation and formation of some compound caused by alternate evacuation and saturation with carbon dioxide.

Analysis of these three possibilities, taking into account the data obtained by multiple saturation with radioactive carbon dioxide and also the solubility of carbon dioxide in the sunflower leaf, makes it seem unlikely that a statistical exchange takes place with a compound completely dissolved

in the sap of the leaf. The other two possibilities appear to be much more reasonable. A choice between these two cannot be made from the data available at the present time.

Tentative estimates of the amount of compound potentially capable of exchanging carbon dioxide in this way yield a value of from 0.004 to 0.005 moles per kilogram of fresh sunflower leaves. In this calculation it was assumed that the reaction takes place between equimolecular quantities of the two reactants.

TABLE IV

COMPARISON OF CARBONATE AND NONCARBONATE FIXATION OF CARBON DIOXIDE BY UNILLUMINATED SUNFLOWER LEAVES
VOLUMES OF CARBON DIOXIDE

INITIALLY ABSORBED	RETAINED ON STORAGE	RETAINED ON ACIDIFICATION
ml.	*ml.*	*ml.*
1.112		0.0302
0.793		0.0367
0.983*		0.0198
1.205	0.329†	
	0.146‡	0.0168
1.807§		0.0841
0.546	0.246	0.0178
0.679	0.322	0.0298
2.733	0.388	0.0226
1.138	0.361	0.0294
1.045	0.463	0.0656

* Initially pretreated with ordinary carbon dioxide.
† Pretreated initially with ordinary carbon dioxide and then saturated with radioactive carbon dioxide, evacuated and swept out once with air.
‡ Same as † but swept out an additional three times with ordinary carbon dioxide.
§ Saturated three times with radioactive carbon dioxide.

In table IV a comparison is made of the amount of radioactive carbon, in terms of carbon dioxide, retained by sunflower leaves after storage and after acidification. The amount retained after acidification (noncarbonate carbon) is roughly 10 per cent. of the carbonate carbon. The amount of carbonate carbon dioxide may be obtained by subtracting the values listed in column 3 from the corresponding values in column 2. The amount of radioactive carbon dioxide measured initially is of little significance because of the differences in the amount of diffusion of the gas from leaves occasioned by the transfer of the leaves to the measuring vessel.

PHOTOSYNTHESIS

One of the chief purposes for investigating the absorption of carbon dioxide by leaves is to determine how carbon dioxide absorbed from the air becomes involved in the photochemical reaction of the photosynthetic process. By the use of radioactive carbon dioxide it has been possible to demonstrate

that leaves can utilize for photosynthesis carbon dioxide which has already been absorbed by the leaves. In this way it has been shown that the absorption of carbon dioxide by leaves for photosynthetic use is independent of the photochemical reaction. Since the photosynthesis of carbon dioxide already contained within the leaf can be followed, and since it is possible to differentiate between different types of combination of carbon dioxide within the leaf, it may be possible to determine which fractions of the carbon dioxide are used in the photosynthetic process.

Sunflower leaves (discs 4.5 cm. diameter) were saturated in the dark with radioactive carbon dioxide at one atmosphere pressure and about 5° C. The discs were removed from the radioactive carbon dioxide and as quickly as possible brought into sunlight. After fifteen minutes of insolation the discs were acidified with hydrochloric acid and the liberated carbon dioxide removed by warming in a vacuum. The radioactivity of the acidified leaf residue and the solution from the insolated leaves was in two experiments 1.49 and 3.03 times the radioactivity of the noninsolated leaves.

Evacuation of leaves after they had been saturated with radioactive carbon dioxide reduced the amount of radioactive carbon fixed by illumination. In one experiment the reduction was from 3.14 to 1.35 arbitrary units. (The units used were the ratios of the radioactivities of the illuminated to unilluminated leaves, measured after acidification.)

Leaves depleted of their easily removable carbon dioxide, by permitting this fraction to diffuse into the atmosphere in the dark, also showed decreased photosynthesis. The amount of radioactive carbon fixed by illumination was less, the longer the time the leaves were stored in the dark (table V).

TABLE V

EFFECT OF CARBON DIOXIDE DIFFUSION ON THE AMOUNT OF PHOTOSYNTHESIS

INTERVAL IN DARK	CARBON DIOXIDE CONTAINED		NONCARBONATE CARBON FIXED		ILLUMINATION
	TOTAL	MOBILE	TOTAL	PHOTOSYNTHESIS	
min.	*ml.*	*ml.*	*ml.*	*ml.*	*min.*
0.0	0.363	0.298	0.0397	0.0370	81
16.7	0.156	0.0911	0.0141	0.0114	25
30.2	0.0981	0.0337	0.0119	0.0091	25
109.8	0.0644	0.000	0.0027	0.000	0
0.0	0.468	0.348	0.0245	0.0147	15
19.0	0.149	0.029	0.0152	0.0054	15
59.4	0.120	0.000	0.0098	0.000	0
0.0	0.620	0.464	0.0392	0.0173	15
17.1	0.173	0.019	0.0247	0.0028	15
69.7	0.154	0.000	0.0219	0.000	0

From these experiments it is apparent that removal of the loosely bound carbon dioxide decreases the amount of photosynthesis, although it is not clear whether the decrease is due to loss of free carbon dioxide or to a diminution of some easily dissociable compound.

RATE OF PHOTOSYNTHESIS.—Rate measurements of the photosynthesis of radioactive carbon dioxide showed that most of the fixation occurred in the first few minutes of illumination (fig. 1). This is not surprising in view of

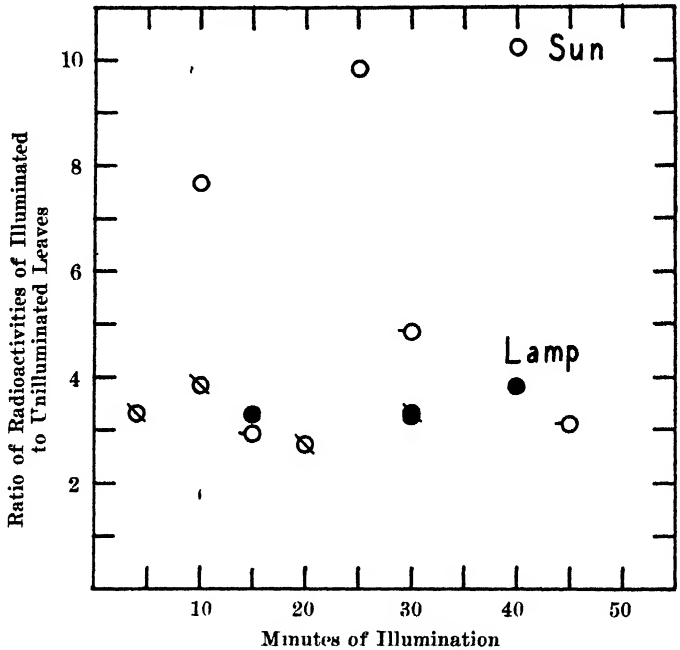

FIG. 1. Rate of photosynthesis of radioactive carbon dioxide contained within the leaf.

Experimental points marked alike belong to the same series of measurements. The points marked with an open circle, ○, were obtained by solar illumination; all other points were obtained by illumination with a 500-watt Mazda lamp.

the dependence of photosynthesis on the content of radioactive carbon dioxide within the leaf. As may be seen from table V this amount decreases very rapidly with time. The rate measurements were made in the following manner.

Discs cut from sunflower leaves (4.5 cm. in diameter) were saturated with radioactive carbon dioxide and then illuminated. The discs were placed on blotting paper which was saturated with water and laid in a brass pan. This pan was set on four supports in another pan of water; this arrangement retarded the loss of water from, and excessive heating of, the

leaves. A glass shield was supported over the leaves in order to leave a considerable space for air circulation. Another glass plate mounted on four tall legs covered the whole assembly. Air blown through the assembly cut down the heating effect and quickly removed any radioactive carbon dioxide which diffused out of the leaves. The results of these experiments are shown in figure 1 where the ordinates are the ratios of the radioactivities of the illuminated to the unilluminated leaves, determined after acidification.

Too much significance must not be attached to the enhanced photosynthesis observed for sunlight as compared to light from a 500-watt Mazda lamp; factors other than increased light intensity were probably involved, as other experiments indicated.

In these experiments the leaves were not deprived of atmospheric carbon dioxide, and it is possible that this carbon dioxide reacted with insoluble radioactive carbonates thus making available radioactive carbon dioxide for photosynthesis. Whether this type of absorption and exchange is operative can be easily determined by future experiments.

The fact that the photosynthetic process can utilize carbon dioxide that has been stored in the leaf for some time must be taken into account when interpretations are made of measurements on such quantities as photosynthetic quotient, quantum yield, and induction periods.

Respiration of newly formed photosynthate

Experiments were carried out to determine whether the carbon newly assimilated in photosynthesis was easily lost through respiration. Discs of sunflower leaves were saturated with radioactive carbon dioxide and then illuminated for a short period of time. The discs were placed in the dark and tested at various intervals for the amount of noncarbonate radioactive carbon they contained. The ratio of this amount to the amount contained by an unilluminated leaf is plotted against time in figure 2. The results show that the loss due to respiration is quite rapid. Whether this rate is greater or less than the rate of loss of carbon from other organic compounds contained in the leaf, *e.g.*, glucose, was not determined.

Summary

1. The investigation of the absorption of carbon dioxide by living sunflower leaves by means of radioactive carbon dioxide has shown that the three possible methods of absorption, previously proposed, are all operative; namely, solution in the water of the sap, reaction with soluble buffer substances, and reaction with insoluble carbonates, presumably calcium carbonate.

2. In addition to these reactions, carbon dioxide reacts to form a noncarbonate derivative of which little is known.

3. The carbon dioxide absorbed previous to illumination can be used in the photosynthetic process. From this it is evident that the absorption of carbon dioxide for photosynthetic purposes is not a part of the photochemical reaction.

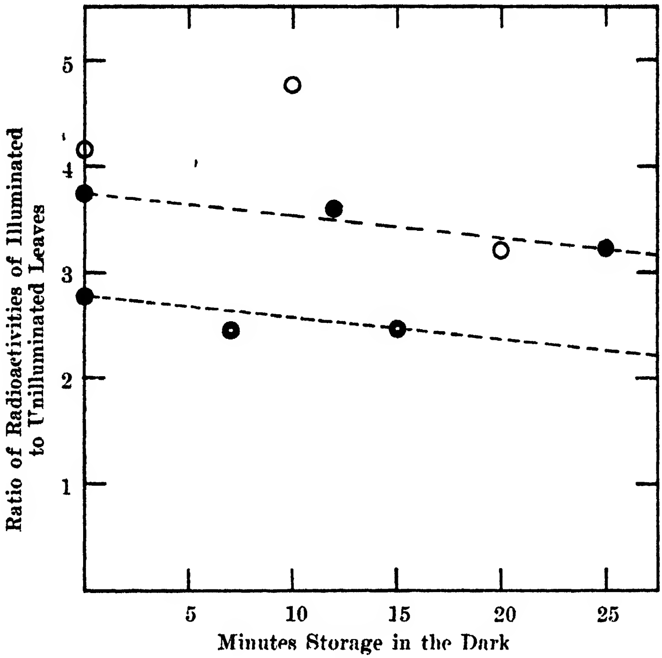

FIG. 2. Rate of respiration of newly formed photosynthate.
Experimental points marked alike belong to the same series of measurements.

4. The newly formed photosynthate is quite rapidly used up in respiration. How its rate of respiration compares with the rate of respiration of other organic compounds contained within the leaf has not been determined.

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NATURE OF BOUND WATER IN COLLOIDAL SYSTEMS

RAY CALVIN CHANDLER

(WITH ONE FIGURE)

Introduction

The state of water in colloidal systems is a controversial subject. Discovery of the colloidal state by GRAHAM (**18**) was followed by extensive studies by VAN BEMMELN (**66, 67, 68, 69**) and ZSIGMONDY (**73, 74**). Thermodynamic studies by KATZ (**36**) indicated significant changes in free energy and heat content in colloidal systems at low water content. OVERTON (**51**) concluded that part of the water in animal tissue did not have normal solvent properties. The work of ROSA (**59**) and HOOKER (**28**) suggested a relationship between certain hydrophilic bio-colloids and cold resistance in plant tissue. NEWTON and GORTNER (**49**) proposed a cryoscopic method for the measurement of hydrophilic colloids as a criterion of the state of water in living and non-living colloid systems. The apparently unusual state of water thus disclosed by a vast amount of data has been called "bound" water. It has been assumed that the presence of colloidal material was responsible for the observed phenomenon. It is of particular importance in biological systems because of certain implications with regard to hardiness and drought resistance in plants and animals. The majority of investigations have dealt with plant physiology.

The estimation of bound water is based upon the physical measurement of a variety of properties of water. Some workers compute the amount of bound water from the total change in thermodynamic activity of the water in a given system while other workers include only that part of the change in thermodynamic activity which cannot be accounted for by osmotically active substances. In this paper the calculations are based upon the latter concept and bound water is defined as the amount of water required to compensate for the deviation of some thermodynamic property of water from the measurement expected in simple solution.

It has been suggested (**17**) that bound water is due to the orientation of water molecules about the colloidal particles and that the water, thus removed from the body of the solution, loses its solvent properties. A few investigators have disagreed with these premises. The data of HILL (**27**) and GROLLMAN (**21**) did not show appreciable amounts of bound water in many colloidal systems. GREENBERG and GREENBERG (**20**) subjected the concept of lost solvent powers to test and found no evidence for bound water on that basis. A knowledge of the nature of the phenomenon observed is necessary for the interpretation of its rôle in biological processes.

Methods for bound water studies

The methods used to measure the properties of water are based on physico-chemical laws applicable to all components of the solution. Attention is centered upon water since it is the major constituent and lends itself readily to measurement. Perhaps the most frequently used methods are based upon the colligative properties of the solution, *i.e.*, vapor pressure lowering, freezing point depression, and osmotic pressure which are interrelated through the applicability of the gas laws to solutions. The chief methods used for the determination of bound water may be classified on the following basis:

I. Colligative measurements.
   - (a) Freezing point depression when solutes are added (**3, 49, 60, 63, 64**)
   - (b) Vapor pressure methods (**2, 15, 16**)
   - (c) Osmotic pressure (**40, 61**)

II. Change of state.
   - (a) Latent heat of fusion (**45, 57, 60, 65**)
   - (b) Volume change (**32, 43, 59, 60**)
   - (c) Heat of vaporization (**21, 27**)

III. Distribution of solutes.
   - (a) Ultrafiltration (**20**)
   - (b) Differential concentration (**44**)

IV. Resistance to pressure (**45, 48, 63**).

Special methods other than the above have been applied to the study of this problem (**1, 15, 22, 23, 29, 42**).

Interpretation of colligative measurements is based on limiting laws for ideal solutions such as might be found in dilute binary solution. The assumption that the thermodynamic contribution of several components in a complex solution is additive is false. The heat of vaporization method introduced by Hill (**27**) directly compared complex solutions and largely obviated this assumption. The difficulties to be met in these studies may be illustrated by the work of Kistler (**38**) and Dexter (**10**). The former concluded that all methods depending upon freezing may involve errors due to non-equilibrium and the latter found irreversibility of ice formation in bio-colloids. Some methods are well suited to a study of the properties of water at high water content while other methods are better suited to systems at low water content.

The problem

This investigation was planned as a study of true and colloidal solutions to disclose the factors responsible for deviations in colligative measurements which are often employed in the estimation of bound water. Any given

colligative determination has a theoretical value based on the ideal gas laws but usually its empirical value differs somewhat from the theoretical. The calculations in this paper are based on the empirical value for a substance dissolved in pure water. A measurement which yields a value greater than expected is called a *positive* deviation while a measurement less than the empirical value is called *negative*. By appropriate sequence of experiments both positive and negative deviations are produced and an attempt is made to evaluate the factors responsible for the deviations.

Methods and materials

Two colligative methods were used in this study on the state of water. The first consisted of vapor pressure measurements made by a highly sensitive static vapor pressure apparatus described in a previous paper (**5**). Gelatin was selected as a typical hydrophilic colloid upon which much data has been secured (**3, 16, 20, 21, 22, 23, 29, 32, 35, 42, 46, 50, 58, 65**). Potassium chloride was selected as a reference solute since its vapor pressure in true solution has been shown to behave in a regular manner over the range of concentrations to be studied (**14**); furthermore its combination with gelatin (**50**) has been found to be negligible.

The second method utilized freezing point data obtained by use of Beckmann thermometer, freezing point tube, air chamber and insulated ice bath. Glucose and KCl were selected as reference solutes. The thermometer was calibrated and kept at low temperature while not in use. Molal freezing point depression-concentration curves were prepared from data (**31**) for each reference substance. The various electrolyte, non-electrolyte, and dipolar solutions were prepared from C.P. standard chemicals. Eastman's de-ashed gelatin was used. The plant sap examined was expressed from plants that had been "hardened" by subjection to low temperature for a period of time.

This application of the cryoscopic method for the determination of bound water differs from the usual method in using two types of solutes (electrolyte and non-electrolyte) as reference substances instead of confining the measurements exclusively to one reference substance.

Theoretical considerations

Vapor pressure constitutes an ideal method for calculation of thermodynamic functions in the study of solutions. In an attenuated atmosphere, as under its own vapor pressure, the vapor of an aqueous solution may be considered as a perfect gas in rapid reversible equilibrium with the liquid phase. The vapor pressure (p) is proportional to the fugacity (f)[1] and the activity (a)[2] is equal to the relative vapor pressure (p/p°). At constant

[1] Fugacity: the vapor pressure of a gas which obeys the ideal gas law.

volume, as under condition of these experiments the free energy (F)[3] and the vapor pressure are related by the equation

$$\triangle F = RT \ln p/p^{\circ} \qquad 1$$

which represents the free energy change involved in the isothermal reversible transfer of one mole pure water to the solution. The differential rate of change of a property with addition of a substance to the solution is called a partial molal quantity. Assuming that the partial molal heat content ($\overline{H}$)[4] is constant over the temperature range studied, its value may be calculated from the relationship

$$\overline{H} = -\frac{RT^2 \, d \ln p^{\circ}/p}{dT}. \qquad 2$$

The change in heat content calculated by this method presumably differentiates between kinetic energy which is a function of temperature and the orienting or binding forces which are independent of temperature. The partial molal entropy ($\overline{S}$)[5] of the water component may be obtained from the relationship

$$\overline{S} = \frac{\overline{H} - \overline{F}}{T}. \qquad 3$$

KATZ (**37**) considers entropy to be a measure of the change in randomness of water molecules in colloidal systems.

The phenomenon of solution is a reflection of interacting forces between components and no one component may be singled out arbitrarily as the cause of the deviations. The properties of the solution are determined by the similarities and dissimilarities of all components. Ideal solutions are rare. The vast majority show deviations from ideal behavior even in binary solution. The energy relations between components of any solution is expressed by the equation (**41**)

$$\frac{N_1 \, (d \ln f_1)}{dN_1} + \frac{N_2 \, (d \ln f_2)}{dN_1} + \frac{N_3 \, (d \ln f_3)}{dN_1} \; . \; . \; . \; . = 0 \qquad 4$$

where N is the mole fraction, f the fugacity, subscripts indicate components; temperature, pressure, and volume are assumed constant. Activity or vapor

[2] Activity: the thermodynamically effective concentration of a constituent rather than the molar concentration.

[3] Free energy: the energy change in a process which may be used to do work; a measure of the tendency of a reaction to go in a given direction. Under conditions of these experiments it equaled the maximum work possible. It also equaled the total energy change less the amount involved in entropy change.

[4] Heat content: the total energy content, in calories, of a gram mole of a constituent. Actually only the change in heat content can be measured. The gain or loss is due to changes in physical properties of the molecule.

[5] Entropy: the energy, in calories per degree, absorbed in a process but not available for work. As used here positive values indicate a more random condition, negative values a less random condition of the molecules.

pressure may be substituted for fugacity in this equation which emphasizes the fact of energy exchange within the solution.

The possibilities of deviations in actual solutions containing ions, non-electrolytes, and dipolar compounds of diverse electrical properties and molecular sizes may well be considered. In a typically non-ideal solution of ions the DEBYE-HÜCKEL theory (**7**, **30**) indicates that under constant conditions of dielectric constant and temperature the activity of the ions is affected by a number of factors. This is shown by the equation

$$-\ln \gamma\ (\text{ions}) = \frac{N^\circ z^2 e^2 b\sqrt{\mu}}{2\,DRT\ (1 - ab\sqrt{\mu})} \qquad 5$$

where γ is the activity coefficient; N° the Avagadro number; z, valence; e, unit charge; D, dielectric constant; T, absolute temperature; μ, a function of concentration and valence; a, ion diameter; while R and b are constants. DEBYE and MCAULAY (**9**) and SCATCHARD (**62**) have applied the concept to non-electrolytes in ionic solutions and found the relation.

$$-\ln \gamma\ (\text{non-electrolyte}) = -\frac{N^\circ e^2 \mu\, \alpha}{DRT\, c} \qquad 6$$

where α, a constant characteristic of the non-electrolyte, has a positive value when the non-electrolyte reduces the value of the dielectric constant, and c indicates the radius of the particle. Equations 5 and 6 show that (in a common environment) concentration, valence, dielectric constant, and temperature have opposite effects upon the activities of electrolytes and non-electrolytes in many cases.

In a two-component solution according to equation 4 $N_1 \frac{(d \ln a_1)}{dN_1} = -N_2 \frac{(d \ln a_2)}{dN_1}$; that is, a change in activity of one component indicates an opposite change in activity of the other component.

In a three component solution which is saturated with respect to the second component, the activity of the second component is fixed and under such conditions $N_1 \frac{(d \ln a_1)}{dN_1} = -N_3 \frac{(d \ln a_3)}{dN_1}$; that is, the change in activity of the solvent reflects a change in activity of the third component. Thus the vapor pressure of an aqueous solution may be utilized to determine energy relations between components, to measure the degree of orientation of water molecules, and to study fundamental differences between kinds of solutes.

Experimentation

The experimental work may be divided into the four following steps:

(1) A vapor pressure study on a two component true solution;

(2) A vapor pressure study on a two component colloidal solution;

(3) A vapor pressure study on a three component colloidal solution;
(4) Freezing point studies on true and colloidal solutions.

Vapor pressure measurements were made on a dilute potassium chloride solution at two temperatures to determine thermodynamic functions of the water component. The data appear in table I and energy data are listed in table IV. Consideration of the data in table IV shows that the heat content and entropy of the water was slightly greater than for pure water.

TABLE I

VAPOR PRESSURE LOWERING IN A DILUTE KCl SOLUTION (0.109 M) AT 1° C. AND 20° C.

	Hg, 20° C.	Hg, 1° C.
	mm.	*mm.*
	0.0614	0.0162
	0.0616	0.0160
	0.0612	0.0159
Mean	0.0614	0.0160

The second experimental solution was an aqueous solution of isoelectric gelatin (3.45 per cent.). It was subjected to vapor pressure measurements at the same two temperatures. The data are listed in table II and values for the thermodynamic functions appear in table IV. A very slight increase in heat content and entropy of the water component is indicated by these data, suggesting that in spite of the lowering of vapor pressure and decreased activity of water in this solution the water molecules actually have slightly greater freedom for random distribution. In accordance with equation 4, the decrease in activity of the water component reflects an increase in activity of the gelatin component, regardless of the fact that its mole fraction is unknown.

TABLE II

VAPOR PRESSURE LOWERING OF A 3.45 PER CENT. GELATIN SOLUTION

	Hg, 20° C.	Hg, 1° C.
	mm.	*mm.*
	0.0013	0.0003
	0.0015	0.0003
	0.0022	0.0003
	0.0018	0.0002
Mean	0.0017	0.0003

The third solution to be investigated was a three component colloidal solution consisting of the previously discussed isoelectric gelatin solution to which was introduced a gas-free charge of KCl producing a 0.069 M solu-

tion with respect to KCl. Vapor pressure data for this solution are tabulated in table III. The vapor pressure lowering due to gelatin is assumed to be constant in solutions 2 and 3. The vapor pressure lowering due to KCl is found by difference. The observed molal vapor pressure lowering $\frac{(P^\circ - P)}{M}$ for KCl in the colloidal solution is compared to that determined from a study of solution 1. Negative deviations are found which also show a marked temperature coefficient. The partial molal functions for water given in table IV show that for this colloidal solution, as in the previous one, the heat content and entropy of the water has been increased slightly by the presence of the colloidal substance. Assuming that the gelatin solutions are saturated with respect to gelatin, the activity of gelatin is unchanged in solutions 2 and 3, therefore the change in molal vapor pressure lowering of KCl shown in table III indicates a change in activity

TABLE III

VAPOR PRESSURE LOWERING OF 0.069 M KCl SOLUTION IN PRESENCE OF 3.45 PER CENT. GELATIN

	HG, 20° C.	HG, 1° C.
	mm.	*mm.*
	0.0384	0.0093
	0.0398	0.0097
	0.0387	0.0093
	0.0406	0.0092
	0.0393	0.0090
	0.0379	0.0093
	0.0382	
	0.0386	
	0.0395	
Mean	0.0390	0.0093
VPL due gelatin	0.0017	0.0003
VPL due KCl	0.0373	0.0090
$\frac{P^\circ - P}{M} = \frac{0.0373}{0.0693} =$	0.5382	$\frac{0.0090}{0.0693} = 0.1299$
$\frac{P^\circ - P}{M} = \frac{(KCl - H_2O)}{(system)} =$	0.5837	0.1540
Ratio $\frac{\text{observed}}{\text{empirical}} =$	0.922	0.844
Percentage deviation	−7.8	−15.6

of KCl as well as water. In each solution the heat content and entropy of the water component is slightly greater than for pure water. Assuming that entropy is a measure of randomness of water molecules it appears that the water molecules are quite as free in any of these solutions as in the pure

solvent. The observed deviation, therefore, is not due to the orientation of water molecules but to the interaction of the components of the solution.

TABLE IV

PARTIAL MOLAL FUNCTIONS OF WATER COMPONENT IN DILUTE COLLOIDAL AND NON COLLOIDAL SOLUTIONS

SYSTEM	$\overline{F}_1$ °C.	$\overline{H}_1$° −20°C.	$\overline{S}_1$ C.
(1) H_2O–KCl (0.109 M)	−1.78	1.74	0.013
(2) H_2O–gelatin (3.45%)	−0.03	0.48	0.002
(3) H_2O–gelatin–0.069 M KCl	−1.00	2.52	0.013

The fourth line of evidence dealt with freezing point studies on both colloidal and non-colloidal solutions of varying complexity. The first series were three-component solutions consisting of water and two reference solutes used in these experiments. The data are shown in table V. The calculated molal freezing point depression refers to the value obtained when the solutes are dissolved in pure water.

TABLE V

DEVIATIONS IN FREEZING POINT DEPRESSION WHEN GLUCOSE IS ADDED TO NON COLLOIDAL SOLUTIONS CONTAINING IONS

SYSTEM	SOLUTE ADDED	MOLAL FREEZING POINT DEPRESSION		DEVIATION
		CALC.	OBS.	
	mols.	°C.	°C.	%
(a) H_2O–0.21 M KCl	0.40 glucose	1.89	1.98	4.8
(b) H_2O–0.42 M KCl–0.40 M glucose	0.40 glucose	1.91	2.12	11.0

When the non-electrolyte glucose was placed in the non-colloidal solution containing ions the freezing point depressions were greater than calculated values and the magnitude of the positive deviations was a function of the ion concentration.

A second type of non-colloidal solution was next examined, using glucose as the reference solute. Aqueous solutions of amino acids are dipolar in nature and constitute important constituents of many natural solutions. When the non-electrolyte was added to these compounds the magnitude and sign of the deviations varied considerably and was apparently a function of length of the carbon chain. The data are given in table VI. The effect of ionic solutions, including KCl, upon the freezing point depression of amino acids may be found in table VIII.

TABLE VI

DEVIATIONS IN FREEZING POINT DEPRESSION WHEN GLUCOSE IS ADDED TO AMINO ACID SOLUTIONS (DIPOLAR COMPOUNDS)

Amino acid	Carbon atoms	Glucose	Molal freezing point depression		Deviation
			Calc.	Obs.	
		mols.	°*C.*	°*C.*	%
0.5 M glycine	2	0.74	1.906	1.861	−2.4
0.5 M alanine	3	0.75	1.907	1.916	0.5
0.5 M valine	5	0.73	1.906	2.000	4.9

The third group examined contained colloids. The positive deviations noted for glucose in the presence of simple solutions of gelatin and hemoglobin (table VII, *a* and *c*) are characteristic of the data usually reported for bound water. The tendency of KCl to give negative deviations in many complex solutions is striking. A sample of plant sap known to contain natural dipolar and ionic compounds yielded a large positive deviation when

TABLE VII

DEVIATION IN FREEZING POINT DEPRESSIONS AS VARIOUS COMPONENTS ARE ADDED TO SOLUTIONS

System	Solute	Molal freezing point depression		Deviation
		Calc.	Obs.	
	mols.	°*C.*	°*C.*	%
(a) H_2O–5% gelatin	0.80 glucose	1.910	2.00	4.7
(b) H_2O–5% gelatin–0.8 M glucose	0.40 KCl	3.315	3.28	−1.1
(c) H_2O–5% hemoglobin	0.69 glucose	1.905	2.07	8.6
(d) H_2O–5% hemoglobin–0.4 M glucose	0.22 KCl	3.375	3.24	−4.0
(e) Wheat sap ("hardened")	0.44 glucose	1.895	2.18	15.1
(f) Wheat sap ("hardened")	0.31 KCl	3.360	3.08	−8.3
(g) H_2O–0.4 M KCl–0.8 M glucose	5% gelatin	1.860	(−0.037)*	
(h) H_2O–0.5 M alanine–0.5 M glucose	5% gelatin	1.860	(−0.015)*	
(i) H_2O–0.5 M glycine–0.74 M glucose	5% gelatin	1.860	(−0.013)*	

* Observed elevation of freezing point which *rose* slightly when gelatin was added.

glucose was used as the reference solute but a negative deviation when KCl was the reference solute.

The foregoing experiments roughly differentiate the effects of typical substances; namely, electrolytes, non-polar and dipolar non-electrolytes, and hydrophilic colloids. Some, and often all, of these substances are components of systems examined for bound water. Each of these might be expected to play a part in this problem owing to structure or electrical properties.

TABLE VIII

INFLUENCE OF ENVIRONMENT IN NON-COLLOIDAL COMPLEX SOLUTIONS UPON THE "NORMAL" BEHAVIOR OF COMPONENTS

GROUP	SOLVENT	SOLUTE ADDED	DEVIATION	METHOD	REFERENCE
	mols.		%		
(A)	0.140 KCl	0.372 M sucrose	5.8	Freezing point	(9)
	0.236 "	0.611 " "	9.5	" "	"
	0.369 "	0.851 " "	15.7	" "	"
	0.467 "	0.756 " "	18.5	" "	"
	0.703 "	0.511 " "	26.9	" "	"
	0.941 "	0.263 " "	34.9	" "	"
(B)	0.335 LiCl	Succinic acid	−12.6	Solubility	(39)
	0.40 NaCl	" "	− 8.4	"	"
	0.67 LiCl	Boric acid	−13.2	"	"
	0.80 NaCl	" "	− 1.5	"	"
(C)	0.50 LiCl	Glycine	8.0	"	(53)
	0.50 NaCl	"	3.9	"	"
	0.50 $NaNO_3$	"	12.2	"	"
(D)	0.50 KCl	Glycine	0.9	"	"
	2.00 KCl	"	− 9.2	"	"
(E)	0.50 NaCl	Leucine	− 9.5	"	"
	0.50 KCl	"	− 8.1	"	"
(F)	0.50 $BaCl_2$	Leucine	13.9	"	"
	0.50 $CaCl_2$	"	17.6	"	"
(G)	0.50 NaCl	Glycine	−14.0	Freezing point	(52)
	0.50 NaCl	Alanine	− 3.0	" "	"
(H)	0.50 $BaCl_2$	Glycine	−28.0	" "	"
	0.50 $BaCl_2$	Alanine	−15.0	" "	"
(I)	0.50 CH_3COOK	Glycine	− 5.2	" "	"
(J)	0.10 KNO_3	Glycine	− 6.8	" "	"
	0.50 KNO_3	"	−22.4	" "	"
(K)	0.01 $ZnCl_2$	Glycine	− 2.6	Potentiometric	(34)
	0.10 "	"	−19.4	"	"
	0.10 "	Valine	− 9.1	"	"
	0.50 "	"	−42.2	"	"

Discussion

The assumption that anomalous changes in activity of a substance in solution are based on chemical combination, association, or dissociation has been convenient; the concept of forces other than these, however, should not be neglected. From a thermodynamic standpoint any change in free energy must be balanced by an equal and opposite change within the system. Vapor pressure measurements have been utilized in this work on relatively simple solutions because of the high accuracy obtainable for very dilute solutions where concentration effects were at a minimum. Utilizing thermodynamic concepts it appears that the activity of water may deviate from

usual values to either greater or less values without materially affecting the randomness of orientation of water molecules.

The negative deviation of vapor pressure lowering of KCl solution in the presence of gelatin indicated that the activity of KCl had been decreased. If this is a fundamental principle it would be reflected in the solubility of a slightly soluble electrolyte in the presence of a colloid. FAILEY (**12**) found the solubility of TlCl in the presence of edestin agreed with that concept. JOSEPH (**35**) also found a decrease of activity of many salts in the presence of gelatin. GORTNER and GORTNER (**17**) and GERIKE (**16**) present data subject to the same interpretation. It seems evident that the changes taking place in the solution as indicated by a measurement of the water are the result of the interaction of all components and not the effect of the colloid alone.

The increase in heat content of colloidal solutions as indicated in the data of table IV is opposed to the concept of "binding" or molecular orientation as a dominant factor in the solution. One should expect orientation to decrease the heat content. The data presented here are supported by the findings of HAMPTON and MENNIE (**22**) and HORN and MENNIE (**29**) for gelatin solutions.

The deviation from additivity of colligative properties in colloidal solutions, which is sometimes used as a measure of the effect of hydrophilic colloids on the state of water therein, may be produced also in non-colloidal solutions as shown in tables V, VI, and VIII. By use of the non-electrolyte in the presence of ions large positive deviations may be produced. By use of a suitable electrolyte in presence of non-electrolytes, particularly certain ones having dipolar properties, large negative deviations may be produced. The concept of non-solvent bound water has no meaning in case of negative deviations such as shown here. A physical interpretation is needed which considers both positive and negative deviations. These deviations are in accord with equations 5 and 6 and experimental work reported in the literature (**9, 40, 52, 53, 54, 55, 56, 62**).

The complex nature of the interacting forces within true solutions may be illustrated by examples from the literature as shown in table VIII. Column 1 groups the table for description; column 2 gives the composition of the solvent; column 3, the kind of solute added; column 4, the deviation as determined by the method listed in column 5. Group (A) illustrates the interaction of ions and non-electrolytes. The interaction of ions and the undissociated parts of weak electrolytes is shown in group (B). The remaining examples portray the interaction between ions and dipolar compounds in which concentration of ions and structure of dipolar substances are shown to play a part. In this category, group (C) represents the behavior of a dipolar substance in the presence of monovalent ions. The effect

of concentration of KCl is exhibited in (D). Group (E) illustrates the effect of structure when a six-carbon acid with the same dipole moment as that in (C) and (D) is introduced into a solution of monovalent ions. Valence and structure effects may be noted by appropriate comparisons in (E), (F), (G), and (H). The influence of ion concentration is shown in (J) and (K) while both ion concentration and structure of dipolar compound may be seen in the last. *These are all non-colloidal solutions.*

It appears that the problem of deviations observed here is characteristic of solutions in general and that the conclusion of Hückel (**30**) regarding electrolyte solutions may be extended to dilute colloidal solutions. This leads to the conclusion: *hydration is by no means as important in determining the characteristics of a solution as we are prone to think; the significance of the observed phenomena lies in the electrical forces which permeate the solution.*

The preceding discussion has dealt with phenomena in dilute colloidal solutions, more or less complex in composition, and has emphasized the relative influence of components other than the colloidal substances. An important part of the literature on bound water has dealt with systems at low water content. Rosenbohm (**58**) found that of the thirty-six calories evolved per gram of gelatin in process of saturation, thirty-three calories were given off in the absorption of 0.24 grams of water. Katz (**36, 37**) showed that a typical sigmoid curve, as represented in figure 1, described the relationship between relative vapor pressure and water content for

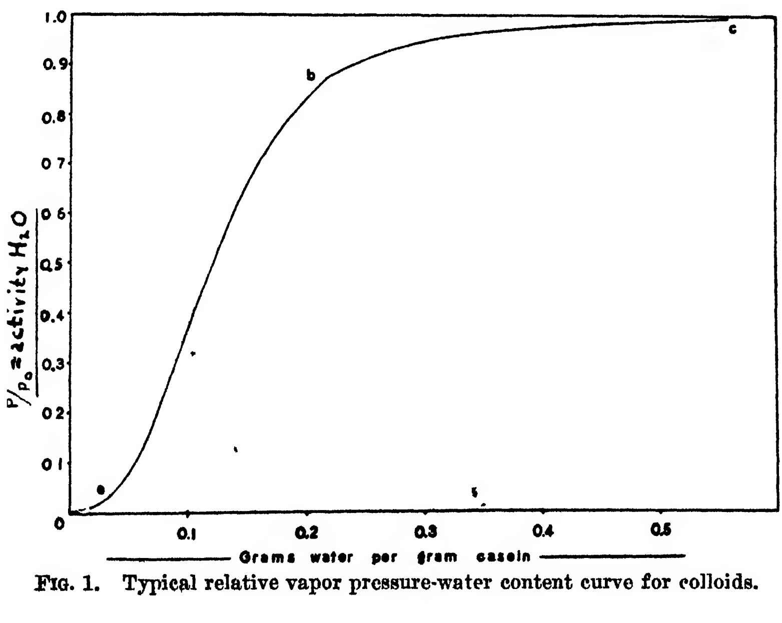

Fig. 1. Typical relative vapor pressure-water content curve for colloids.

many elastic gels. FRICKE and LÜKE (15) made a vapor pressure study on casein and agar at low water content. Values for partial molal free energy ($\bar{F}$), heat content ($\bar{H}$) and entropy ($\bar{S}$) calculated from their data are presented in table IX. An important decrease in entropy below the point *b* on the curve is characteristic. An interesting comparison of energy relations for water in colloids at low water content and for water contained in crystal hydrates may be made from tables IX and X. Above a water content of 0.4 or 0.5 grams per gram colloid, the water in colloidal solutions appears to behave approximately as in dilute true solutions. Similar values for bound water of this type have been found by diverse methods (**1, 22, 23, 29**). The great differences between heat content and entropy of one region and these same functions for the other region suggest that the water molecules are subjected to quite different forces in these two regions. It is justifiable to differentiate two classes of phenomena: first, those that occur in solutions wherein the process approaches reversibility; secondly, those that occur in colloidal solutions at low water content where the process is irreversible. In the first of these it appears that the forces

TABLE IX

ENERGY RELATIONS IN COLLOID SYSTEMS

GRAMS WATER PER GRAM COLLOID	SYSTEM	$\bar{F}$	$\bar{H}$	$\bar{S}$	REFERENCE
gm.					
0.059	H_2O agar	−2214.0	−3436.0	−4.48	(15)
0.125	"	−1177.0	−2502.0	−4.85	"
0.157	"	− 957.0	−2023.0	−3.90	"
0.366	"	− 160.0	− 540.0	−1.39	"
0.478	"	− 60.0	− 75.0	−0.05	"
0.049	H_2O casein	−1267.0	−2297.0	−3.77	"
0.143	"	− 369.0	−1063.0	−2.54	"
0.322	"	− 31.0	− 110.0	−0.29	"
0.437	"	− 8.0	− 28.0	0.13	"
27.96	H_2O gelatin	0.3	4.6	0.04	Table IV
27.96	H_2O-gelatin KCl	0.2	11.6	0.02	Table IV

are predominantly electrical forces existing between components of the solution which tend to make it non-ideal. In the second case one deals with water in a condition similar to that of a crystal hydrate.

The evidence indicates that the chief influence upon the thermodynamic activity of water in a complex colloidal solution is the osmotic force which is not included in the definition used here for bound water. It is indicated that the electrical forces existing between the components of the solution which make it non-ideal are responsible for the phenomena often considered

as a measure of water "bound" by colloids. Under certain conditions the nature of the reference solute may profoundly affect the results obtained in measurements. In a colloidal system of low water content the thermodynamic properties of the water differ from that of the pure solvent.

TABLE X

ENERGY RELATIONS OF WATER IN CRYSTAL HYDRATES

MOL FRACTION H_2O	SYSTEM	$\overline{F}$	$\overline{H}$	$\overline{S}$	REFERENCE
mols					
0.333	$CuSO_4 \cdot H_2O$	-2004	-2783	- 2.61	(70)
0.691	$CuSO_4 \cdot 5H_2O$	- 654	-3051	- 8.04	(4)
0.706	$CdSO_4 \cdot 8/3H_2O$	- 165	-1732	- 5.26	"
0.777	$MgSO_4 \cdot 7\ H_2O$	- 365	-4036	-12.18	"

Summary

Measurements of the vapor pressure lowering and freezing point depression of some true and colloidal solutions were made and the thermodynamic behavior of these solutions has been considered.

A new interpretation of some bound water phenomena has been presented.

Results indicate that bound water in colloidal systems may be of two kinds:

(a) A small amount of water intimately associated with the colloid and having special thermodynamic properties, and

(b) A hypothetical amount of water estimated to account for the differences between the value of a property of water as found in a complex solution and the corresponding value in simple solution.

The second kind of bound water may be exhibited by any complex solution, colloidal or non-colloidal, whose components have properties which make the solution non-ideal.

The part played by colloids in this phenomenon appears to be due chiefly to their electrical properties and may be of minor importance compared with crystalloidal components.

Solvent molecules in a dilute colloidal solution show no reduction in the random orientation characteristic of the pure solvent.

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UNEQUAL DISTRIBUTION OF SOLUBLE SOLIDS IN THE PULP OF CITRUS FRUITS[1]

E. T. Bartholomew and Walton B. Sinclair

(WITH SIX FIGURES)

Introduction

The nature of the citrus fruit is such that it affords many interesting problems of research for the plant physiologist. The pulp of the citrus fruit, unlike that of most edible fruits, is divided into segments (carpels) the walls of which are not readily permeable; and each segment is composed of hundreds of units (vesicles, juice sacs) the walls of which are still more impermeable, especially laterally (**18**).

In the past, investigators have drawn many conclusions that were based entirely on results obtained by analyzing a whole plant or the major portion of a plant. While the results of such analyses have their value, they obviously omit many interesting and vital details. The results reported in this paper illustrate the value of making detailed analyses of the different tissues of a given portion of a plant. They are a part of the results of a line of research which is being carried on at the present time in this laboratory. This concerns the effects of rootstocks, maturity, insecticides, and the physiological disorder "granulation" on the composition of citrus fruit.

Materials and methods

Valencia and Navel oranges of different sizes and of different stages of maturity were used in these tests, primarily; but a few grapefruit were tested also. All mature fruits were peeled, the segments isolated, and the juice from each stem and stylar segment half, or from each entire segment, was tested for the components mentioned in later sections of this paper. When a fruit was too young to be peeled and to have the segments isolated, the entire fruit pulp was bisected transversely. The peel of such fruits was first cut away with a knife, care being taken to leave a minimum of the peel and to cut away a minimum of the pulp.

Juice was extracted from the whole or halved segments of the mature fruits with a modified, old-style lemon squeezer. From the pulp halves of the young fruits, the juice was extracted by subjecting each half to a pressure of 6,000 pounds in a hydraulic press. Aliquots of the juice were centrifuged for three minutes at 1,700 r.p.m. before being tested.

Total soluble solids were determined with an Abbé refractometer. Total acidity was determined in terms of citric acid by titrating with standardized

[1] Paper no. 423, University of California Citrus Experiment Station.

solutions of NaOH, with phenolphthalein as indicator. The pH values were determined with a Beckman glass electrode pH meter.

Sugar determinations were made by the HAGEDORN and JENSEN method (**13, 14**) as modified by BLISH (**4, 5**). The strength of the reagents employed by BLISH was satisfactory for determining the reducing and total sugars as glucose, when the values ranged from 3 to 10 mg. in 10 ml. of citrus juice. The samples were diluted when necessary, so that the values fell within this range. This method was used because comparative tests showed that it was more rapid than the best of the copper reduction methods and, at the same time gave accurate results. Because potassium ferricyanide is subject to gradual change on standing, the glucose factor was redetermined just before making each group of tests. The values were determined by checking against a sample of glucose furnished by the National Bureau of Standards.

Results[4]

SOLUBLE SOLIDS IN CARPELLARY SEGMENTS OF CITRUS FRUITS

SOLUBLE SOLIDS IN STEM AND STYLAR HALVES OF SEGMENTS.—SUTHERST (**20**), CHACE and CHURCH (**6**), BAKER (**2**), and HAAS and KLOTZ (**12**), who made an extensive study of polarity gradients, found that the concentration of soluble solids is noticeably greater in the stylar end of certain mature citrus fruits than in the stem end. This was especially true in the Valencia orange.

As is well known, other conditions being equal, the higher the concentration of soluble solids in a liquid, the lower the temperature required to freeze it. Therefore, in cutting many thousands of mature Valencia oranges during the study of granulation [a physiological disorder of Valencia fruits (**3**)], it was surprising to find that during the previous winter one or more segments in some of the fruits had been frozen at the stylar end and not at the stem end.

In order to find an answer to this apparent anomaly, an effort was made to correlate this condition with the distribution of soluble solids in the juice of the stem and stylar ends of the fruit during the winter months before the fruit was mature. Valencia and Navel oranges and also a few grapefruits were tested. This study entailed the making of almost 3,000 refractometric readings in addition to a large number of acid, sugar, and other determinations. Most of the fruits were tested on the day they were picked or on the following day.

In the localities from which the different fruits were obtained, the Valencias mature from the last of March to the first of May; Navels, from the middle of November to the latter part of December; and grapefruit, from the last of April to the first of June. All of these citrus fruits set at approximately the same time; therefore Valencias and grapefruit mature in

12 to 14 months and Navels in 8 to 9 months. The dates of maturity have an important bearing on the interpretation of the data to be presented.

The results of determining the total soluble solids in separate segment halves of Valencias, Navels, and grapefruits are presented in table I. These

TABLE I

DISTRIBUTION OF SOLUBLE SOLIDS IN STEM AND STYLAR HALVES OF CARPELLARY SEGMENTS OF CITRUS FRUITS IN DIFFERENT STAGES OF MATURITY

DATE FRUIT WAS PICKED AND TESTED	APPROXIMATE AGE OF FRUIT	NUMBER OF FRUITS	NUMBER OF SEGMENTS	PERCENTAGE OF SEGMENTS		
				TOTAL SOLUBLE SOLIDS HIGHER IN STEM THAN IN STYLAR HALVES	TOTAL SOLUBLE SOLIDS HIGHER IN STYLAR THAN IN STEM HALVES	TOTAL SOLUBLE SOLIDS EQUAL IN STEM AND STYLAR HALVES
Valencia oranges						
	mo.			%	%	%
1937						
Nov. 1, 2	6.0	8	81	47	44	9
Dec. 27, 28	8.0	12	131	22	63	15
1938						
Jan. 5, 7, 8	8.0	9	95	25	63	12
Jan. 14–17	8.5	5	51	20	68	12
Feb. 4, 16	9.0	12	121	7	87	6
Mar. 11, 18	10.5	12	120	0	99	1
Mar. 21	11.0	12	129	1	99	0
June 28	14.0	10	106	0	100	0
1939						
Jan. 18	8.5	14	138	4	95	1
Navel oranges						
1937						
Oct. 25–28	6.0	12	130	31	53	16
Dec. 22	8.0	6	63	0	97	3
1938						
Jan. 10, 11, 31	8.5	8	76	1	99	0
Feb. 2, 9, 23, 24	9.5	7	69	0	99	1
Grapefruit						
1938						
Jan. 12–14	8.5	6	71	9	78	13
Feb. 14, 28	9.5	3	39	0	97	3

data show that, as a rule, the more mature the fruit, the greater the percentage of segments in which the total soluble solids were higher in the stylar than in the stem halves. For example, only 44 per cent. of the segments of the Valencias picked November 1 and 2, 1937, (6-month-old fruits), showed a concentration of total soluble solids higher in stylar than in stem halves, whereas practically 100 per cent of the segments of the fruits picked from

March 11 to June 28, 1938 (10- to 14-month-old fruits), showed this condition. Similar trends were exhibited by the Navels and grapefruits.

The ages of the fruits given in table I are only approximate. The setting and maturing dates may be as much as a month or six weeks earlier or later one year than another. For instance, the Valencias tested January 14–17, 1938, were less mature than those tested January 18, 1939. This was shown, not only by the fact that on the latter date a larger percentage of the stylar halves of the segments contained the higher concentration of soluble solids (table I), but by the fact that these fruits had a considerably higher concentration of soluble solids (data not included in table) than those tested January 14–17, 1938.

From the data (table I) one may logically conclude that the concentration of total soluble solids in the stylar half of the citrus fruit does not noticeably exceed that in the stem half until the fruit is nearing maturity. It should be pointed out, however, that even in the youngest of the Valencias tested (6-month-old fruits), 44 per cent. of the segments had a higher concentration of total soluble solids in stylar than in stem halves, and 9 per cent. had equal concentrations in both halves. A similar condition existed in the Navel orange segments.

The diagrams in figures 1, 2, 3, and 4 illustrate the differences in concentration of total soluble solids in segments of Valencias about five months before maturity (fruit no. 103, fig. 1) and about two months after maturity (fruit no. 84, fig. 2), and in Navels about two months before maturity (fruit no. 92, fig. 3) and about two months after maturity (fruit no. 47, fig. 4). These two Valencias and two Navels showed the greatest extremes of all the fruits tested. Valencia no. 103 (fig. 1) had only one segment (no. 6) which

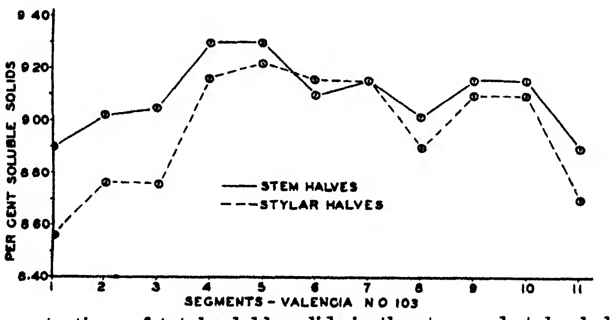

FIG. 1. Concentrations of total soluble solids in the stem and stylar halves of carpellary segments of an immature Valencia orange. With two exceptions, the concentrations are higher in the stem halves.

had a higher concentration of total soluble solids in its stylar than in its stem half. Segment 7 had the same concentration in both halves (9.16 per cent.), but all the other segments had lower concentrations in the stylar than

in the stem halves. Navel no. 92 (fig. 3) showed a condition similar to that in Valencia no. 103 (fig. 1), except that four segments (nos. 1, 2, 9, and 10)

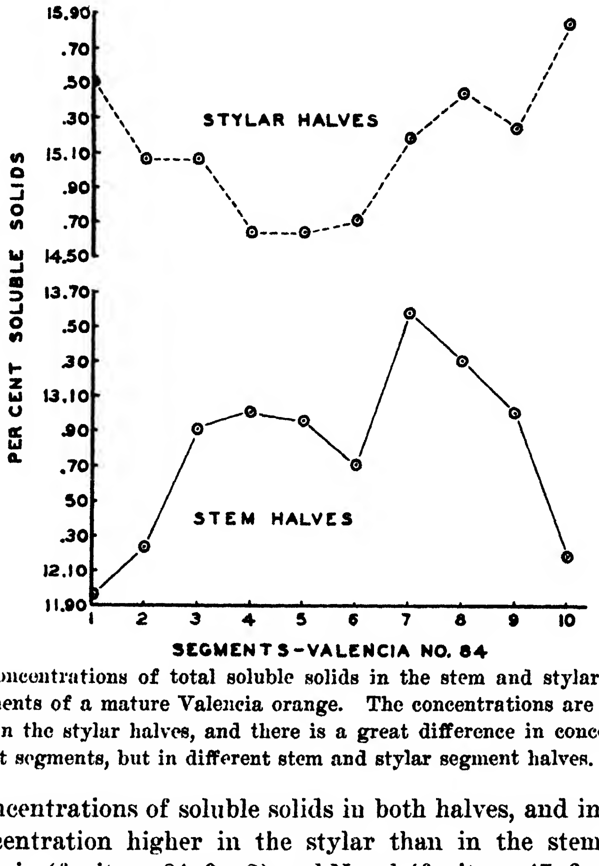

FIG. 2. Concentrations of total soluble solids in the stem and stylar halves of the carpellary segments of a mature Valencia orange. The concentrations are much lower in the stem than in the stylar halves, and there is a great difference in concentrations, not only in different segments, but in different stem and stylar segment halves.

had equal concentrations of soluble solids in both halves, and in no segment was the concentration higher in the stylar than in the stem half. The mature Valencia (fruit no. 84, fig. 2) and Navel (fruit no. 47, fig. 4) not only showed great differences in concentration in different stem and stylar segment halves, but showed much greater concentrations in all stylar halves than in stem halves. In the mature Valencia, segment 10 showed the great-

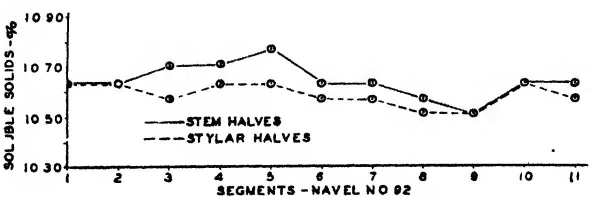

FIG. 3. Concentrations of total soluble solids in the stem and stylar halves of the carpellary segments of an immature Navel orange. The concentrations are higher in the stem halves of all segments except 1, 2, 9, and 10, in which they are equal in the two halves.

est difference between the concentration of soluble solids in the stem and stylar halves; the concentration in the stem half was 12.18 per cent. and that in the stylar half, 15.84 per cent., a difference of 3.66 per cent. A comparison of halves of segment 2 of the mature Navel showed the concentration in

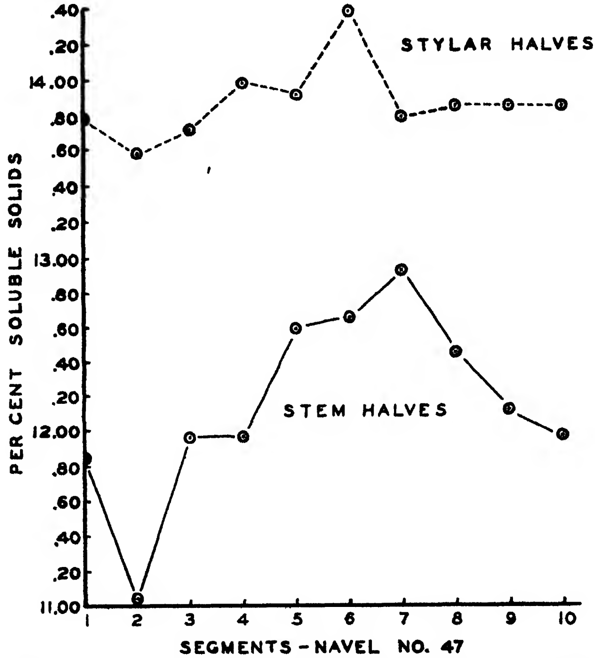

FIG. 4. Concentrations of total soluble solids in the stem and stylar halves of the carpellary segments of a mature Navel orange. As in the Valencia orange (fig. 2), the concentrations in the stylar halves are considerably higher than those in the stem halves; but the concentrations in the two halves of this orange are a little less than in those of the Valencia orange.

the stem half to be 11.04 per cent. and that in the stylar half, 13.58 per cent.; a difference of 2.54 per cent.

Of all the Valencia segments tested, the one having the greatest concentration of soluble solids in the stem half over that in the stylar half was from fruit no. 135, picked January 18, 1938. In this segment the concentration in the stem half was 8.70 and in the stylar half, 7.93 per cent; a difference of 0.77. The average difference for all Valencia segments that had the higher concentration in the stem halves was 0.22 per cent. The segment having the greatest concentration in the stylar half over that in the stem half was from fruit no. 84 (fig. 2), picked June 2, 1938. The average difference for all Valencia segments that had the higher concentration in the stylar halves was 0.76 per cent.

The concentration of soluble solids in Navel segments showed a stem-half high of 12.11 per cent. and a stylar-half low of 11.31 per cent. for all segments having the greater concentration in the stem halves, a difference of 0.80 per cent. The average difference for these segments was 0.17 per cent. The stem-half low for all segments having the greater concentration in the stylar halves was 11.04 per cent. and the stylar-half high, 13.58 per cent; a difference of 2.54. The average difference for these segments was 0.33 per cent. The results of tests of grapefruit segments were similar but are not reported here because only a comparatively few fruits were tested and these were practically all of the same age. It should be borne in mind that the differences and averages given for Valencias and Navels refer to both immature and mature fruits.

One might expect to find that in immature fruits the concentration of soluble solids would be about equal in the stem and stylar halves of the segments, even though there is a much greater concentration in the stylar halves by the time the segments mature. Table I shows that there were comparatively few segments in which the concentration of soluble solids was equal in the two halves. On the other hand, the results of a limited number of tests on whole citrus fruits indicate that in the earlier stages of development the concentration of soluble solids, though not greatly, may be measurably higher in the stem half than in the stylar half Of 13 Valencias about three and one-half months old, 9 had an average of 0.59 per cent. higher concentration of soluble solids in the stem halves than in the stylar halves of the pulp, 1 had equal concentrations in the two halves, and 3 had an average of 0.31 per cent. higher concentration in the stylar halves than in the stem halves. Of 23 Navels of similar age, 21 had an average of 0.23 per cent. higher concentration in the stem halves than in the stylar halves, and 2 had equal concentrations in the two halves. None of the 23 Navels had a higher concentration in the stylar half than in the stem half of the fruit.

The original records show that of those fruits in which the concentration of soluble solids was higher in the stem halves than in the stylar halves of the whole fruits, some had (*a*) the higher concentration of both acids and sugars in the stem half; others had (*b*) acids higher in the stem half and sugars higher in the stylar half; or (*c*) acids higher in the stem half and sugars equal in both halves; or (*d*) acids equal in both halves, but sugars higher in the stylar half; or (*e*) acids higher in the stylar half, but sugars equal in both halves. The last condition mentioned, (*e*), indicates that soluble solids other than acids and sugars are partially responsible for the conditions mentioned (*a*) to (*d*), especially in immature fruits.

When the comparative concentrations of total soluble solids in the stem and stylar segment halves were determined (table I), the total acids (in terms of citric acid) and total sugars (in terms of glucose) were also deter-

mined in the two halves of some of the segments. Because of the great amount of work that would have been involved, not all segment halves were tested for acids and sugars. The tests were made on the stem and stylar halves of 37 segments from both immature Valencias and immature Navels and on stem and stylar halves of 20 segments from mature Navels.

The approximate ages of the fruits from which these segments were taken and the results of the tests are given in table II. The data on total soluble solids are from the stem and stylar halves of whole fruits, while the data on acids and sugars are from segment halves.

TABLE II

AVERAGE PERCENTAGE OF TOTAL SOLUBLE SOLIDS IN FRUIT HALVES AND DISTRIBUTION OF ACIDS AND SUGARS IN CARPELLARY SEGMENTS OF IMMATURE VALENCIA ORANGES AND OF IMMATURE AND MATURE NAVEL ORANGES

Fruits tested	Average total soluble solids in fruit halves		Percentage of segments					
			Acids (in terms of citric acid)			Sugars (in terms of glucose)		
	Stem halves	Stylar halves	Higher in stem than in stylar halves	Higher in stylar than in stem halves	Equal in stem and stylar halves	Higher in stem than in stylar halves	Higher in stylar than in stem halves	Equal in stem and stylar halves
	%	%	%	%	%	%	%	%
Immature Valencias (8–9.5 months old)	10.22	10.54	91	9	0	18	81	1
Immature Navels (6 months old)	9.86	9.90	60	30	10	26	58	16
Mature Navels (9.5 months old)	12.29	13.30	9	91	0	0	100	0

Although the first two lots of fruit mentioned in table II were immature, the average total soluble solids was slightly higher in their stylar halves than in their stem halves. It may be stated, however, that even in these fruits, 27 per cent. of the segments had a slightly higher concentration of soluble solids in the stem than in the stylar halves. The higher concentration in the stylar halves of the last lot of Navels mentioned in the table was to be expected because the fruits were mature.

The data in table II show that although the acid content was higher in 91 and 60 per cent., respectively, of the stem segment halves of the immature Valencias and immature Navels, total sugars were higher in 81 and 58 per cent., respectively, of the stylar segment halves of the same fruits. Of the mature Navels, 91 per cent. had the higher acid content and 100 per cent. had

the higher sugar content in the stylar segment halves. These and other data in the table show that as a result of shifting or of some other process, there is a pronounced difference in the distribution of soluble solids in immature and in mature citrus fruits.

SOLUBLE SOLIDS IN ENTIRE SEGMENTS.—The differential response to low temperatures was not confined to the stem or stylar halves of the different segments. Many fruits were found in which an entire single segment had been frozen and had collapsed, whereas the other segments were unaffected. The apparent explanation for this condition was found by refractometric determinations of the total soluble solids in individual segments of many Valencia and Navel fruits.

In making approximately 1,500 tests, it was found that comparatively great differences may exist between the concentrations in the segments of a given fruit. For example, in a mature Valencia fruit (no. 75) picked June 28, 1938, one segment contained 16.11 per cent. soluble solids, while the two adjacent segments contained 13.41 and 15.44 per cent., respectively. Another segment in a different portion of the same fruit contained only 12.61 per cent.—a difference of 3.50 per cent. between the highest and lowest concentrations in the segments of that fruit.

In the stem half of one of the segments in this fruit (no. 75) the concentration of soluble solids was only 11.38 per cent., while in the stylar half of one of the other segments, the concentration was 16.98 per cent., a difference of 5.60. The differences mentioned for this fruit were greater than for any other fruit tested, but almost all the other fruits were less mature than this one.

SOLUBLE SOLIDS IN NORTH AND SOUTH SEGMENTS OF FRUITS.—Each fruit, before it was picked, was marked to designate its north or south side as it hung on the tree. It was also marked to indicate from which side of the tree it was taken and whether it was an inside or an outside fruit.

When the tabular data on concentration of total soluble solids in different segments was examined an interesting, but as yet unexplained, phenomenon was discovered. Of fruits picked from the outside of the trees and about 4 to 6 feet from the ground, 80 out of 88 Valencias, 25 out of 33 Navels, and 8 out of 9 grapefruits (or 87 per cent. of the 130 fruits) had a higher total soluble-solids content in their three north segments than in their three south segments. This condition held whether the fruits were borne on the north, south, east, or west sides of the trees, provided they were outside fruits.

Figure 5 shows the comparative concentrations of total soluble solids in each of the segments of 3 of the 80 Valencia oranges that showed a higher concentration in their three north than in their three south segments. The vertically hatched columns represent the three north, and the stippled

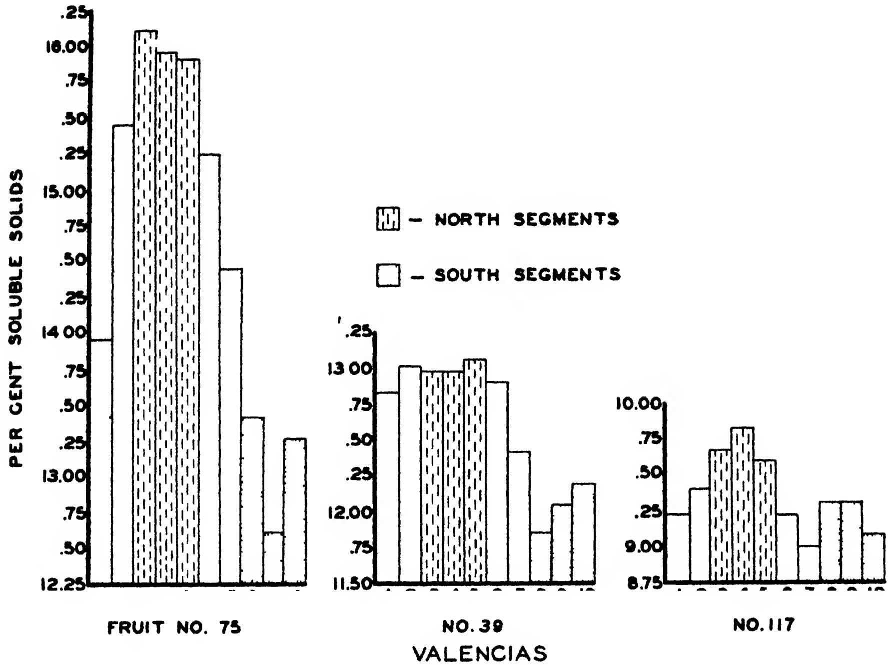

FIG. 5. Comparative concentrations of total soluble solids in the three north and the three south carpellary segments of three Valencia oranges. This figure shows the great difference in concentrations in the different segments of a given fruit.

columns, the three south segments in each fruit. The average total soluble solids for the three north segments of Valencia no. 75 was 15.98 per cent. and for the three south segments, 13.09 per cent. Averages for the three north and the three south segments of fruits nos. 39 and 117 were 12.98 and 12.02 per cent. and 9.69 and 9.23 per cent., respectively. Valencia no. 75 was picked June 28; no. 39, February 12; and no. 117, December 28. The indications are that the more mature the fruit, the greater is the difference in concentration of soluble solids in the segments, and, also, the greater is the north-south polarity of concentrations in the fruit.

Figure 6 represents the comparative total soluble solids in each of the segments of 3 of the 25 Navel oranges that showed a higher concentration in their three north than in their three south segments. The differences, however, were not so great in the Navels as in the Valencias. Navel no. 89 (fig. 6) was more mature than Valencia no. 117 (fig. 5), and Navel no. 46 (fig. 6) was less mature than Valencia no. 75 (fig. 5). Navels nos. 46, 33, and 89 were picked February 24, February 9, and October 27, respectively. The average total soluble solids for the three north segments of Navel no. 46 was 14.39 per cent. and for the three south segments, 12.91 per cent. Aver-

ages for the three north and the three south segments of Navels nos. 33 and 89 were 12.20 and 11.71 per cent. and 11.00 and 10.66 per cent., respectively.

While in 80 out of 88 Valencias and in 25 out of 33 Navels the three north segments contained greater concentrations of soluble solids than the three south segments, attention should be called to the fact that the segment which contained the highest concentration was not always on the north (fruit no.

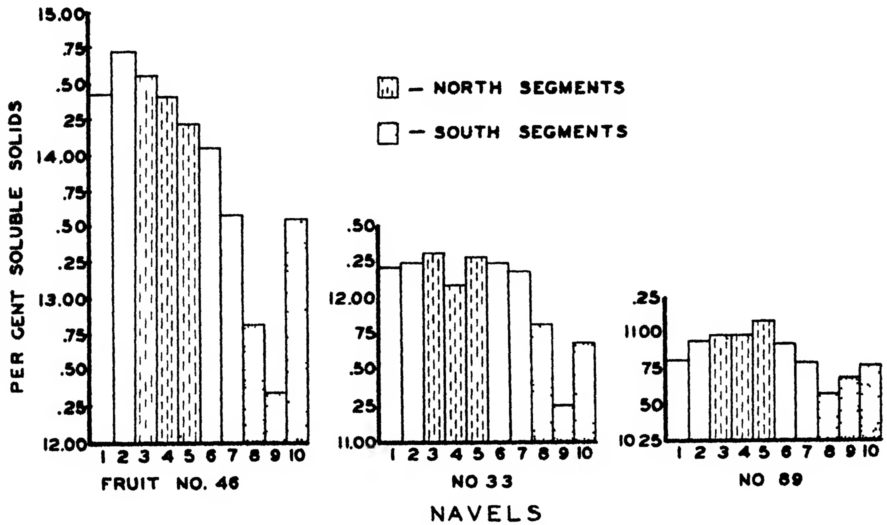

FIG. 6. Comparative concentrations of total soluble solids in the three north and the three south segments of three Navel oranges. The segment differences are similar to those in the Valencias (fig. 5), but are a little less.

46, segment 2, fig. 6), and the one that contained the lowest concentration was not always on the south side of the fruit (fruit no. 117, segment 7, fig. 5). As a rule, however, the concentration of soluble solids in the two segments adjacent to the group of three north segments was higher than that in the two segments adjacent to the group of three south segments. Figures 5 and 6 illustrate this condition.

The north-south polarity concentrations were determined in only six inside fruits, all Valencias, time not permitting the making of more such tests. Two of the six fruits showed higher concentrations of soluble solids in the three north segments than in other segments, and four showed higher concentrations in the three south segments. The exposures of inside fruits are variable; for some fruits may be heavily shaded, while others may receive almost as much light as those on the outside.

Just what significance lies in the fact that, as a rule, the north half of an outside citrus fruit contains a higher concentration of soluble solids than its south half, can be determined only by further study. The fact is interesting

from a physiological viewpoint, but with the data at hand, the condition is as difficult to explain as it is interesting.

SIZE OF SEGMENTS AND CONCENTRATION OF SOLUBLE SOLIDS.—Even by a casual observation it can be determined that the segments in the pulp of a citrus fruit are not all equal in size. Some segments are comparatively large; others may extend the entire length of the pulp, but be only about a fourth to a half as thick as the largest; and in occasional fruits, especially in navels and grapefruits, a segment may extend only a half or two-thirds of the length of the fruit and be very thin. The results of the tests showed that there is no direct relation between the size of the segment and the concentration of total soluble solids which it contains.

SOLUBLE-SOLIDS CONCENTRATION AND THE FREEZING POINT OF CITRUS JUICE

Since the concentration of soluble solids in the stylar halves of many segments of immature and newly matured citrus fruits was equal to, or lower, than that in the stem halves of the same segments (table I, figs. 1 and 3), it is not surprising that freeze injury was found in the stylar as well as in the stem halves. Nor is it surprising that freeze injury was found in one segment of a given fruit and not in another when, as shown in figures 2, 4, 5, and 6, there was such a difference in concentration of soluble solids in the different entire segments. These conditions prevail in the fruit at the time of the year when it is most likely to be subjected to freezing temperatures. Later, as already stated and as found by other investigators (**2, 6, 12, 20, 22**), the concentration of soluble solids is noticeably higher in the stylar half than in the stem half of the fruit; and it seems logical to conclude that if the fruit were subjected to freezing temperatures at this time, the injury would probably register first in the stem half.

The concentration of the total soluble solids in expressed sap does not, alone, determine the exact temperature at which this sap would have frozen while present in the tissues. Articles by other investigators in recent years have presented much data on this point, but they will not be reviewed here. Time and equipment were not available when these tests were made, to determine the freezing point of the juice *in situ* in the segments or segment halves.

A few tests were made, however, to determine whether the concentration of soluble solids in the juice expressed from immature and mature citrus-pulp tissues would give a reliable clue to the freezing-point trends of the expressed juice. The results of these tests are shown in table III. The fruits were not frozen before the juice was extracted. The peels were cut away from the immature Navels, as explained previously; the pulps were bisected transversely, and each half was subjected to 6,000 pounds pressure. The mature Valencias were bisected transversely without peeling, and the

TABLE III

SOLUBLE SOLIDS IN JUICE AND FREEZING-POINT DEPRESSION OF JUICE FROM STEM AND STYLAR HALVES OF IMMATURE NAVEL AND MATURE VALENCIA ORANGES

FRUIT NUMBER	SOLUBLE SOLIDS IN JUICE		FREEZING-POINT DEPRESSION OF JUICE	
	STEM HALF OF FRUIT	STYLAR HALF OF FRUIT	STEM HALF OF FRUIT	STYLAR HALF OF FRUIT
Navel oranges (immature)				
	%	%	°C.	°C.
1	9.07	8.95	0.989	0.980
2	9.01	8.80	0.979	0.963
3	9.15	8.95	1.001	0.997
4	9.61	9.15	1.026	1.006
5	9.61	9.35	1.048	1.019
6	9.77	9.77	1.079	1.034
7	9.35	9.35	0.966	0.962
8	9.50	9.43	0.974	0.994
9	9.69	9.49	0.981	0.967
10	9.50	9.33	0.976	0.859
11	9.89	9.43	0.968	0.960
12	9.07	8.80	0.978	0.955
Valencia oranges (mature)				
13	16.44	19.66	1.935	2.512
14	15.06	17.46	1.863	2.200
15	14.06	16.27	1.590	1.828
16	16.29	17.58	2.055	2.379
17	15.24	17.44	1.780	2.084
18	16.25	17.51	1.902	2.118
19	14.90	16.70	1.719	2.010
20	15.11	17.69	1.809	2.287

juice was reamed from each half. Soluble-solids and freezing-point determinations were made on the juice thus obtained from the two lots. The Navels used in these tests were about 4 months old, and the Valencias, about 17.5 months old. Valencias were used because no mature Navels were available at the time these tests were made.

The data in table III show that the concentration of soluble solids was slightly higher in the stem than in the stylar halves of all the immature fruits except nos. 6 and 7, in which soluble solids were equal in the two halves. The freezing points were also slightly higher for the juice from the stem halves of all the fruits except no. 8.

The extreme differences in concentrations of soluble solids and freezing-point depressions for the juice from the immature Navels were small, 1.09 per cent., and 0.175° C., respectively; but if the differences between the stem and stylar halves are considered, they appear to be significant, since the results by the two methods show the same trends. On the other hand, when the relationship between the soluble solids and the freezing-point depres-

sions is considered without reference to the differences in concentrations in the stem and stylar halves of the fruit, some discrepancies are evident. For example, the juice from the stem halves of fruits 4 and 5 had the same percentage of soluble solids but different freezing-point depressions.

The results of the tests on the mature Valencias (table III) show not only a marked increase in concentration of soluble solids over that of the immature Navels, but also concentrations much greater in the stylar than in the stem halves of the fruits. As would be expected, these increased concentrations resulted in much greater depressions of the freezing points. Here again small discrepancies may be found, however, and a straight line would not be obtained if the percentages of soluble solids were plotted against the freezing-point depressions.

The apparent discrepancies which appear in the results of these tests (table III) may have been caused by experimental error and by soluble inorganic constituents which had a marked effect on the freezing-point depression and but little effect on the refractive index of the solution. Or they may have been caused by the presence of colloidal material which registered in the refractive index but had little or no effect on the freezing-point depression.

As was previously stated, in earlier tests fruits were found in which freeze injury was evident in the stylar and not in the stem halves of the segments. These findings, together with those given in table III, indicate that if immature Navels are subjected to the minimum temperature which will cause injury, the stylar halves of the segments will be injured first.

Color differences in centrifuged juice

The supernatant portion of the centrifuged juice from the two halves of a segment did not always have the same depth of color. Data of this kind were kept on the segments of only 80 of the fruits tested, and most of these segments were from mature or nearly mature fruits. Eighteen of the fruits showed no difference in the color of the juice from the stem and from the stylar halves of the segments. Of the 654 segments of the other 62 fruits, 33 (5 per cent.) had darker juice in the stylar halves than in the stem halves; 328 (50 per cent.) had the darker juice in the stem halves; there was no appreciable difference, however, in the color of the juice from the halves of the remaining 293 segments, There were 5 fruits in which the juice from the stem half of every segment was darker than that from the stylar half.

Differences in juice color were more prevalent and more noticeable in mature than in immature fruits and were found in all three varieties of citrus fruits tested—Valencia, Navel, and grapefruit. In addition to color there were often similar differences in the juice from the whole segments of a fruit.

The degree of any color contrast was not accurately determined by the use of photoelectric or similar equipment, but was determined simply with the unaided eye. Where there was a color difference in the juice from different portions of mature fruits, it appeared that the amount of carotene present might be the controlling factor. In immature fruits the juice was pale gray with a greenish tinge. Contrasts in juice of this kind appeared to be owing to differences in opacity rather than in color.

As yet, no attempt has been made to isolate the substance or substances that are responsible for the color differences, nor is any special significance attributed at this time to their existence. Their significance, if any, and their explanation await further and more accurate tests. These data are included merely to give added evidence of the desirability and value of studying small portions of a given plant.

Discussion

The refractometer was used for determining the dry weights of the citrus juices, expressed in terms of total soluble solids, because preliminary tests showed that the results obtained by this method were as accurate as those obtained by the dry-weight method and were much more rapidly determined. To test the dry-weight method, 25-ml. aliquots of juice were dried in vacuum at 72 to 73 cm. and at a temperature of 55° C. The results of the preliminary tests are given in table IV. In three of the five tests, the results obtained

TABLE IV

COMPARISON OF PERCENTAGES OF SOLUBLE SOLIDS IN CITRUS JUICE, DETERMINED BY DRYING IN VACUUM AND WITH REFRACTOMETER

EXPERIMENT NUMBER	SOLUBLE SOLIDS IN JUICE	
	DETERMINED BY DRYING IN VACUUM*	DETERMINED WITH REFRACTOMETER
1	11.70	11.83
2	11.76	11.91
3	11.58	11.57
4	9.34	9.33
5	10.10	10.26

* Twenty-five-ml. aliquots of juice dried in vacuum at 72 to 73 cm. and at temperature of 55° C.

with the refractometer are slightly higher than those obtained by the dry-weight method. It is not likely that the differences are significant, but if they are, it is probable that the results obtained with the refractometer are the more accurate because of the time element and the effect of the vacuum on the results given by the dry-weight method. The results compare very satisfactorily with those of GORTNER and HOFFMAN (**10**), who used the refractometer for the determination of moisture as well as for the determina-

tion of colloidal material in expressed plant saps. Therefore, the determination of the soluble solids by the refractive index of citrus juice is considered to be a rapid and an accurate method for determining its dry weight.

An unequal distribution of soluble solids has been found in fruits other than citrus. LALL (17) and ARCHBOLD and BARTER (1) have shown that the most highly colored half of an apple, when cut from stem to calyx, and the calyx half of the transversely cut apple, may contain a higher concentration of soluble solids than the opposite halves. HARDING (15) found the concentration of soluble solids was highest in the skin of the Jonathan apple, with a gradual decrease toward the pith. TUCKER (21) and SCOTT (19) found an unequal distribution of soluble solids in different varieties of melons.

It is possible that migration or redistribution of soluble solids in such fruits as apples and melons could be more easily explained than similar processes in citrus fruits. The segment (carpel) walls in citrus fruits, especially the walls of the vesicles which contain the soluble solids, have a high degree of impermeability. REED (18) used different dyes on fresh vesicles and on those that had been treated with such substances as alcohol, ether, NaOH, and HCl and concluded that the lateral walls of the vesicles contained suberin or cutin, which made them comparatively impermeable. He found that the walls of the stalk and of the distal tip of the vesicle were more permeable than those of the body of the vesicle. From these results it would appear that to migrate from the stem end to the stylar end of the fruit, soluble solids would have to pass out of each vesicle stalk, in which there is no developed vascular tissue, through the segment wall, and into the surrounding parenchymatous tissue of the peel. From there they would have to migrate slowly through this tissue toward the stylar end of the fruit or would have to find their way to one of the bundles of the peel, where they would be transferred more rapidly. All vascular bundles of the citrus fruit are separated from the walls of the segments by at least several layers of parenchymatous tissue, except where they pass through the inner walls and into the ovules.

That the segment and vesicle walls of citrus fruits are comparatively impermeable is further indicated by the work of CURTIS and CLARK (8), who found that when the two opposite sides of apples and tomatoes were kept at different temperatures, there was a movement of water, in vapor form, from the warm to the cool side. No such movement of water could be induced in oranges and potatoes by the same treatment. For a more extended discussion of the work on apples, see CURTIS (7). It may be mentioned here that similar tests had been made on oranges at the Citrus Experiment Station before it was known that CURTIS and CLARK had done their work. The results were the same as those obtained by these men. The nature of the tissues in

the pulp of the orange is such that the movement of water is probably prevented largely by the comparative impermeability of the walls rather than merely by the absence of air spaces, as CURTIS and CLARK concluded.

Several other theories might be advanced to explain the presence of a higher concentration of soluble solids in the stylar than in the stem end of mature citrus fruits. For example, there may be an uneven rate of respiratory activity in the two ends of the fruit as it matures, or the soluble solids may naturally continue to flow in greater abundance to the tissues that have been most recently in a meristematic condition. The navel portion of the Navel orange contains a higher concentration of soluble solids than any other portion of the mature fruit, and it is the youngest portion of the Navel fruit.

Inasmuch as there is no transfer of substance from one segment to another and each segment behaves principally as a separate unit, it is not surprising that one segment may have a higher concentration of soluble solids than another, even the adjoining one, in the same fruit. This condition fits in very well with the theory that each carpellary segment of the pulp of a citrus fruit represents a modified leaf, because no two leaves on a twig contain the same concentration of soluble solids. It is not the purpose of this paper, however, to present any argument for or against any theory concerning the ontogeny of citrus or other fruits. For recent discussions of three different theories on this subject, see EAMES and MACDANIELS, (**9**); HAYWARD, (**16**); and GREGOIRE, (**11**).

Sufficient data are not yet available to explain the north-south polarity of concentration of soluble solids in citrus fruits. That the different amounts of light received by the two sides of the fruit may be a factor is indicated by the fact that the difference in concentrations in the north and south halves of the fruit is usually greater in the fruits from the south than in those from the north side of the tree. Just why the shaded side of the fruit on the south side of the tree and the exposed side of the fruit on the north side of the tree should have the highest concentration is difficult to explain, unless one concludes that the south side of the south, east, and west fruits receive more direct light and, therefore, more heat, and that the resulting increase in respiration causes a decrease in total concentration of soluble solids in the south half over that in the north half of the fruit. This could not be true for the north fruit however, unless one were to conclude that the south half, though shaded, was warmer than the north half.

A study of the vascular system of the citrus fruit does not afford any direct explanation for the condition under discussion. A transection through the center of the fruit shows as many vascular bundles in the center of the fruit as there are segments (carpels). Each bundle, as a rule, lies near the inner point of contact of each two adjacent segment walls. Branches from these bundles pass into surrounding parenchymatous tissue

and into the ovules, but they do not pass through or even contact the segment walls except at the point of attachment of the ovules. One large bundle may be seen in the mesocarp (spongy parenchyma of the peel) in the vicinity of the center of the external wall of each segment; another, in a similar position but in the depression opposite the juncture of two segment walls. Branches from these main bundles pass into all parts of the mesocarp of the peel, but they do not penetrate or contact the tissues of the segment walls. The lack of direct contact of any of the vascular bundles with any of the vesicles, or even with the segment wall which surrounds the vesicles, would at least lead one to question the theory that the differences in concentration of soluble solids in different segments or parts of segments, are due to the difference in amounts of food or food materials supplied by the different vascular bundles of the fruit. It is probable that an explanation would more likely be found by a study of conditions inherent in the segments themselves.

Summary

Mature oranges and grapefruit have a considerably higher concentration of total soluble solids in their stylar than in their stem halves. Immature fruits may have equal amounts in both halves or may have the higher concentration in their stem halves. The latter condition suggests an explanation for the fact that the stylar half of an immature fruit segment may be injured by low temperature while the stem half may remain uninjured.

The concentration of total soluble solids in the segments of a given fruit may differ greatly, especially if the fruit is mature. This may be largely because of the comparatively impermeable nature of the walls of the segments and juice vesicles.

Of 130 fruits tested, 87 per cent. had a higher concentration of total soluble solids in their three north than in their three south segments. This condition was found whether the fruits were borne on the north, south, east, or west side of the tree, provided they were exposed, outside fruits.

Apparently the concentration of soluble solids in a segment of a citrus fruit is not governed by the size of the segment.

The total soluble-solids content of expressed citrus juice, determined with the refractometer, appears to be a reliable index to the temperature at which such juice will freeze.

In the citrus fruits tested, the color of the juice was not the same in all the segments of a given fruit, nor was the color always the same in the stem and stylar halves of the segments.

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TOLERANCE OF SHORTLEAF PINE SEEDLINGS FOR SOME VARIATIONS IN SOLUBLE CALCIUM AND H-ION CONCENTRATION[1]

A. G. CHAPMAN

(WITH FIVE FIGURES)

Introduction

Shortleaf pine, *Pinus echinata* (Mill.), is known to be limited in its natural distribution to low-calcium, acid soils. The species occurs principally on residual sandstone and shale soils and on acid, cherty soils, low in soluble basic compounds, derived by weathering from highly siliceous limestones. A few areas in the Central States region have high-calcium, acid soils which have been derived from calcium sulphate, or gypsum. Such areas are small and relatively unimportant in the portion of the region within which shortleaf pine occurs. Failures of seedlings at and soon after emergence have been experienced in attempts to produce planting stock in newly established forest nurseries containing neutral or alkaline soils high in soluble calcium. In other nurseries, failures have been attributed to accumulation of basic compounds in the soil, resulting from sprinkling with alkaline water having a high calcium content. Apparently, the adverse effects of high-calcium neutral or alkaline soils on the seedlings are restricted to the early stages of seedling development; when seedlings of plantable size developed under favorable nursery soil conditions have been transplanted to neutral or alkaline field soils with relatively high calcium, they have survived and grown quite acceptably.

Apparently little attention has been given to shortleaf pine's specific ranges of tolerance for soluble calcium and hydrogen-ion concentration. ILLICK and AUGANBAUGH (5), in studies on pitch pine, *Pinus rigida* (Mill.), observed that "for best results, it should have acid soil in the nursery. If there is an excess of lime in the soil, seedlings turn yellow. . . . Pitch pine . . . almost never occurs naturally on limestone soils. . . . It is able to make good growth when planted thereon. . . . An excess of lime in the soil creates conditions which are most favorable for the activities of damping-off fungi. . . . Under ordinary conditions, (seedlings) would occur there naturally, were they able to survive damping-off during the first six months after germination." They did not mention any direct effects of excess of lime upon the seedlings. After investigations on ponderosa pine, *P. ponderosa* (Dougl.), HOWELL (4) concluded that "calcium is not dele-

[1] A contribution from the Central States Forest Experiment Station, U. S. Forest Service, Columbus, Ohio.

terious to the seedlings. The fact that the plant does not assimilate the ion in alkaline soils may be of more importance." He found indications that seedlings of the species grow best in an acid medium, but that under field conditions other factors may have more influence on growth than the soil reaction.

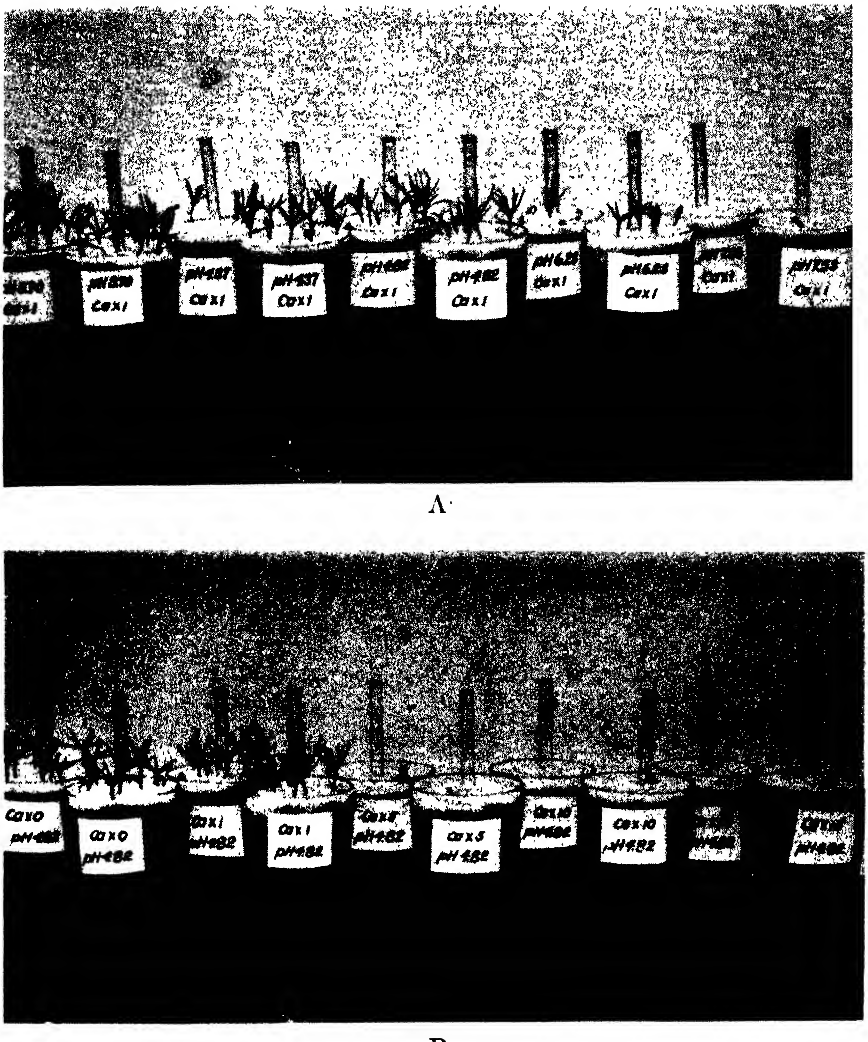
The purpose of the present investigation was to determine whatever direct effects soluble calcium and hydrogen-ion concentration may have upon young seedlings of shortleaf pine and the ranges of tolerance of the seedlings for soluble calcium and hydrogen-ion concentration. The experiments were conducted in the greenhouse of the Department of Botany, Ohio State University, in the spring of 1937, and in the Forest Service nursery near Chillicothe, Ohio, in the summer of that year.

Materials and methods

Two 5-unit series of cultures of shortleaf pine, each in duplicate (fig. 1), were set up in average-sized drinking glasses under greenhouse conditions and maintained for 18 days. The culture media of series A had a common level of calcium, 249 p.p.m., in solution but had pH values of 3.7, 4.4, 4.8, 6.2, and 7.5; those of series B had a common pH value of 4.8, but their calcium levels were no calcium (except possible impurities in C.P. compounds) and 249, 1,245, 2,490, and 3,735 p.p.m. The excessive amounts of calcium were employed because at the time of the experiment no indication of seedling tolerance for calcium was available.

Aside from differences in the culture solutions, the several units were similar in construction. In each glass 250 ml. of washed quartz sand had been placed, and in the center of each was a glass watering tube, held upright by the sand and extending to the bottom. The sand in each glass was watered with 80 ml. of a culture solution. Twelve shortleaf pine seeds which had begun to germinate between layers of moist cheesecloth were distributed over the sand surface and covered with 30 ml. of dry sand. The quantity of culture solution was sufficient to bring the "solution table" to a point 1¼ inches below the surface and to maintain the surface layer in a moist—but not waterlogged—condition.

The stock solutions used were modifications of the buffered solutions used by Hopkins and Wann (3) for culturing *Chlorella* sp. Their composition is indicated in table I. The amount of calcium used in any stock solution is indicated in terms of the quantity contained in the "balanced" culture solution, solution A. Solution A and the phosphate buffer solutions are identical with those used by Hopkins and Wann. Solution B contains no calcium, and contains nitrogen in the form of ammonium nitrate. Solutions C, D, and E have nitrogen added in the form of ammonium nitrate and calcium in the form of calcium acetate. Table II indicates in columns



A

B

FIG. 1. Cultures of shortleaf pine seedlings, each in duplicate: A, in uniform water-soluble calcium and varied H-ion concentration; and B, in uniform H-ion concentration and varied water-soluble calcium.

3 to 6 the quantities and combinations of the solutions listed in table I which were used to obtain the final culture solutions.

The original solution-table levels were uniformly maintained throughout the two series, by daily adding distilled water through the watering tube. Only the initial 80 ml. of culture solution was applied to any unit during the experiment. After 18 days, none of the pH values had changed more

TABLE I

STOCK SOLUTIONS FROM WHICH CULTURE SOLUTIONS WERE DERIVED

Stock solution	Composition	
Solution A—(Ca × 1)	$Ca(NO_3)2 \cdot H_2O$	2.95 gm.
	$MgSO_4 \cdot 7\ H_2O$	0.40 gm.
	$Fe_2(SO_4)3$	0.005 gm.
	Distilled water to	1,000 ml.
Solution B—(Ca × 0)	NH_4NO_3	2.00 gm.
	$MgSO_4 \cdot 7\ H_2O$	0.40 gm.
	$Fe_2(SO_4)3$	0.005 gm.
	Distilled water to	1,000 ml.
Solution C—(Ca × 5)	$Ca(C_2H_3O_2)2 \cdot H_2O$	10.296 gm.
	NH_4NO_3	2.000 gm.
	$MgSO_4 \cdot 7\ H_2O$	0.400 gm.
	$Fe_2(SO_4)3$	0.005 gm.
	Distilled water to	1,000 ml.
Solution D—(Ca × 10)	$Ca(C_2H_3O_2)2 \cdot H_2O$	21.580 gm.
	NH_4NO_3	2.000 gm.
	$MgSO_4 \cdot 7\ H_2O$	0.400 gm.
	$Fe_2(SO_4)3$	0.005 gm.
	Distilled water to	1,000 ml.
Solution E—(Ca × 15)	$Ca(C_2H_3O_2)2 \cdot H_2O$	32.984 gm.
	NH_4NO_3	2.000 gm.
	$MgSO_4 \cdot 7\ H_2O$	0.400 gm.
	$Fe_2(SO_4)3$	0.005 gm.
	Distilled water to	1,000 ml.
Phosphate buffer solutions		
1	0.1333 mol. H_3PO_4	13.07 gm. per liter.
2	0.1333 mol. KH_2PO_4	18.156 gm. per liter.
3	0.1333 mol. K_2HPO_4	23.23 gm. per liter.

than 0.2 unit. Seed and seedling mortalities for the several cultures during the 18 days, represented in figure 1, were used as the basis of judging the effects of the various gradations of calcium content and hydrogen-ion concentration. Differences among them were tested for significance by Chi square (χ^2).

To observe any possible plasmolysis of cells, fresh root-tip sections from seedlings a few weeks old grown in acid soil media were placed in hollow ground slides for 2 hours and then examined under a microscope (1) in a series of solutions with a pH value of 4.8, ranging in calcium content from 249 to 1,245 parts per million, (2) in the solution of series A having a pH value of 6.23, as presented in table II, and (3) in distilled water. The series of solutions, the calcium content of which ranged from 1 to 5 times the amount in the "balanced" solution, were employed in order that the point of plasmolysis, if any, might be determined rather definitely. Simultaneously, germinating seeds were placed on a series of filter papers saturated with the several solutions for observation of any toxic effects.

Osmotic values of the solutions in which tests were made for plasmolytic

TABLE II

MIXTURES OF SOLUTIONS LISTED IN TABLE I TO OBTAIN THE FIVE LEVELS OF pH AND OF CALCIUM IN CULTURE SOLUTIONS

INITIAL pH (ELECTRO-METRIC)	CALCIUM	SOLUTION AND VOLUME	0.133 MOL. H_3PO_4	0.133 MOL. KH_2PO_4	0.133 MOL. K_2HPO_4
Series A	*p.p.m.*	*ml.*	*ml.*	*ml.*	*ml.*
3.70	249	A–125	10	115.00	.
4.37	249	A–125		125.00	
4.82	249	A–125		121.88	3.13
6.23	249	A–125		87.50	37.50
7.53	249	A–125		25.00	100.00
Series B					
4.82	0	B–125		121.88	3.13
4.82	249	A–125		121.88	3.13
4.82	1245	C–125		121.88	3.13
4.82	2490	D–125		121.88	3.13
4.82	3735	E–125		121.88	3.13

action on root cells were determined by the cryoscopic method. For comparison with these values, osmotic values of a solution of alkaline nursery soil in which germinating seed had died and of the expressed saps of roots and tops of seedlings from the same lot were obtained.

To determine possible effects of the hydrogen-ion concentrations of soil media upon those of sap of shortleaf pine seedling roots, pH values of expressed saps from seedling roots grown in soils of different acidity were determined.

Measurements of the buffer capacities of two lots of expressed root sap of seedlings a few weeks old were made on 10-ml. samples by titration against N/10 solutions of HCl and NaOH. The roots used were from two lots of seedlings grown in a single nursery bed in an alkaline soil having a pH of 7.1 and an acid soil having a pH of 5.6. Only a few yellow-green seedlings still survived in the alkaline soil. Drops of N/10 HCl were thoroughly mixed with a sap sample, one by one, until the pH was lowered to approximately 3.5, pH being recorded after the addition of each drop. Similarly, a N/10 NaOH solution was mixed with a sap sample until the pH value was increased to 8.2, pH being recorded after the addition of each drop. The results of the titrations were plotted on semilogarithmic paper, pH values on the logarithmic scale and volume of added solutions on the arithmetic scale (fig. 2).

In an effort to increase the buffer capacity of the cell sap of roots of seedlings growing in nursery soil, superphosphate was applied to plots of seedlings developing in a section of a bed where the soil had a pH value of 5.5. Other plots of seedlings in the same section of bed were not treated. After a month, roots of seedlings from the fertilized plots and from the

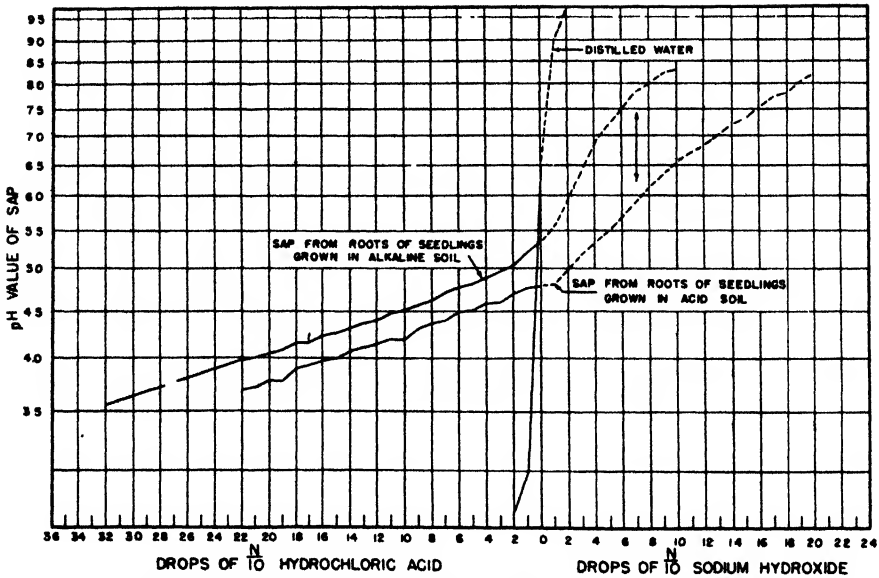


FIG. 2. Results of titration of two lots of sap expressed from roots of shortleaf pine seedlings grown in acid (pH 5.6) soil and in alkaline (pH 7.1) soil against N/10 HCl and N/10 NaOH. Trends of curves indicate relative buffer capacities.

unfertilized plots were lifted and tested for buffer capacity in the manner described for tests on seedlings growing in acid and alkaline soils. Colorimetric phosphorus tests were made on samples of expressed sap from the two lots of seedling roots.

Amount of water-soluble calcium was determined on the nursery soils which had proved toxic to seedlings and on those which had not, for comparison with the amounts of soluble calcium in the culture solutions indicated in table II.

Results

Greenhouse sand cultures

The results of the sand cultures are presented in figure 1 and in table III. Figure 1, A, represents the results of the tests on shortleaf pine seed and seedlings in culture media with five levels of pH and uniform calcium at the end of 18 days. Original pH is indicated on each container, varying from 3.70 through 4.37, 4.82, and 6.23 to 7.53. It may readily be observed that no notable effect was produced upon seedling behavior by successively higher degrees of alkalinity up to and including pH 4.82. In the pair of cultures having a pH of 6.23, seedling growth rate was low and mortality was high. In the culture medium with pH 7.53, 18 of the 24 seeds continued to germinate until emergence, but all seedlings died. When the

behavior of seedlings in cultures having pH values of 6.23 and 7.53 is compared with that of seedlings in the three cultures having lower values (table III, series A), it is clear that the differential was greater in mortality of seedlings than in mortality of germinating seed. It should not be overlooked, however, that germination was lower in the high-pH media than in the low. In regard to series A, the hypothesis was that the behavior of both seed and seedlings in all cultures did not differ from that of the control culture, with pH 4.82. The Chi-square (χ^2) tests indicated that only the seed and seedlings in cultures having pH values of 6.23 and 7.53 behaved in a way differing significantly from that of the control.

TABLE III

CHI SQUARE (χ^2) TESTS OF SIGNIFICANCE OF DIFFERENCES* IN BEHAVIOR OF SEED AND SEEDLINGS IN UNIFORM CALCIUM AND VARIED H-ION CONCENTRATION AND IN UNIFORM H-ION CONCENTRATION AND VARIED CALCIUM

SERIES A. UNIFORM CALCIUM (249 P.P.M.), VARIED H ION CONCENTRATION

pH VALUE	SEED			SEEDLINGS			
	SURVIVING TO EMERGENCE	NOT SURVIVING	SIGNIFICANCE BY χ^2 TEST	TOTAL OBSERVED	SURVIVING AT 18 DAYS	NOT SURVIVING	SIGNIFICANCE BY χ^2 TEST
	number	*number*		*number*	*number*	*number*	
4.82†	22	2		22	14	8	
3.70	24	0		24	18	6	
4.37	21	3		21	13	8	
6.23	18	6	‡	18	4	14	§
7.53	18	6	‡	18	0	18	§

SERIES B. UNIFORM H-ION CONCENTRATION (pH 4.82), VARIED CALCIUM

CALCIUM LEVEL	SEED			SEEDLINGS			
	SURVIVING TO EMERGENCE	NOT SURVIVING	SIGNIFICANCE BY χ^2 TEST	TOTAL OBSERVED	SURVIVING AT 18 DAYS	NOT SURVIVING	SIGNIFICANCE BY χ^2 TEST
p.p.m.	*number*	*number*		*number*	*number*	*number*	
1 ×†	22	2		22	14	8	
0 ×	22	2		22	15	7	
5 ×	15	9	§	15	0	15	§
10 ×	14	10	§	14	0	14	§
15 ×	7	17	§	7	0	7	§

* Determined by YATE's method (1, table VIII). ‡ = significant at the 5 per cent. level.
† Control hypothesis.
§ = highly significant at the 1 per cent. level.

Figure 1, B, represents results of tests in culture media with five levels of soluble calcium and a common pH level of 4.82. Responses of germinating seed and of seedlings in cultures containing no added calcium and

in cultures containing 249 parts per million of added calcium were closely parallel. In each of these pairs of cultures, 22 of the 24 planted seed germinated and 34 per cent. of the seedlings died. Chi-square tests indicated that behavior of seed and seedlings was not significantly different for these two cultures, but that the mortality in each of the three cultures with higher calcium was significantly greater than that in the control culture (table III, series B).

Relative osmotic pressures

In table IV are given the corrected freezing-point depressions and calculated osmotic pressures of (1) 11 culture solutions, containing 1 to 5 times as much calcium as the "balanced" solution; (2) a calcareous nursery soil solution; and (3) the expressed sap of the roots and tops of young seedlings grown in acid soil. The pressures for all the culture solutions are lower than that for the expressed sap of the roots, 5.30. The alkaline soil solution, with a pH value of 7.8, had an osmotic pressure of only 0.42, a value closely approximating those for agricultural soils. The osmotic pressures of expressed saps of roots and top have the general relation characteristic for most plants, a top-root ratio of more than 1:1.

TABLE IV

Freezing-point depression and osmotic pressure of culture solutions, highly calcareous soil, and cell sap of roots and tops of young shortleaf pine seedlings

Material	Freezing-point depression (corrected)	Osmotic pressure
Culture solution	° *C.*	*atm.*
a. pH 4.8, Ca × 1	−0.265	3.19
b. pH 6.2, Ca × 1	−0.320	3.85
c. pH 4.8, Ca × 1.45	−0.330	3.97
d. pH 4.8, Ca × 1.90	−0.335	4.03
e. pH 4.8, Ca × 2.36	−0.340	4.09
f. pH 4.8, Ca × 2.81	−0.350	4.21
g. pH 4.8, Ca × 3.27	−0.355	4.27
h. pH 4.8, Ca × 3.72	−0.360	4.33
i. pH 4.8, Ca × 4.18	−0.360	4.33
j. pH 4.8, Ca × 4.63	−0.370	4.45
k. pH 4.8, Ca × 5.00	−0.380	4.57
Calcareous soil solution (pH 7.8)	−0.115	0.42
Expressed seedling sap		
Root	−0.440	5.30
Top	−0.810	9.75

None of the culture solutions listed in table IV plasmolyzed cells of sections of young seedling rootlets submersed in them for 2 hours. This was expected, on the basis of the relative osmotic pressures of expressed root sap and the solutions. After 48 hours, however, it was evident that germi-

nating seed on filter papers saturated with the solutions containing more calcium than the "balanced" solution had been injured.

Buffer capacities of expressed saps

The buffer capacities determined on expressed root saps are presented graphically in figures 2 and 3. Figure 2 shows the relative buffering against change in pH of lots of sap from young seedling roots grown in acid (pH 5.6) and alkaline (pH 7.1) nursery soils. The generally parallel curves with closely similar gradients to the left of the zero ordinate indicate that there was little difference in the buffering of the two samples of sap against an acid. The reading on the glass-electrode potentiometer at the point of 22 drops of added acid solution indicated a 0.3 greater change for the sap having the higher pH value. This difference may not be significant. Both saps appeared well buffered in contrast with distilled water. When titrated with the N/10 alkali, the saps responded not only more quickly but differently; 6 drops of the alkali produced a change in pH of 2.1 in the less acid sap, but a change of only 0.9 in the more acid sap. The divergence of the curves must be observed in the vertical direction of the double arrow. The flattening off of the curves at their outer extremities was probably caused by buffering effects of sodium salt accumulation.

It may be noted that the pH value of the sap from seedlings produced

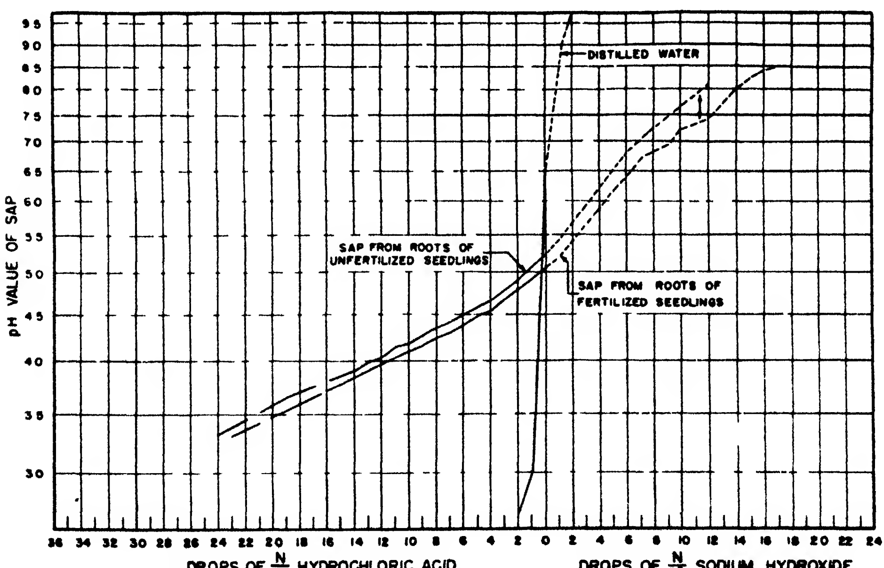


Fig. 3. Results of titrating cell sap expressed from roots of phosphorus-fertilized and unfertilized short leaf pine seedlings against N/10 HCl and N/10 NaOH. In each case, the nursery soil had a pH value of 5.5. Trends of curves indicate relative buffering.

in alkaline soil was 5.4 and that of the sap from seedlings produced in acid soil was 4.8. While these two values have undoubtedly been affected in part by the corresponding soil-medium values, they do not equal the latter, which indicates a degree of buffering.

The buffer capacity of the cell sap of seedlings was somewhat increased by adding superphosphate to the soil. Colorimetric tests revealed a somewhat higher phosphorus content in the expressed sap of fertilized plants than in that of unfertilized plants. Little difference in buffering could be distinguished between the saps of fertilized and unfertilized seedlings when they were titrated against acid (fig. 3). A difference between these saps in buffering was indicated, however, when they were titrated against alkali. This differential is manifest in the pronounced divergence of the two graphs as viewed properly along lines parallel to the ordinate. Application of 12 drops of N/10 NaOH produced a change in pH value of +2.3 in sap from fertilized seedlings and one of +2.8 in sap from unfertilized seedlings, a difference of 0.5.

Water-soluble calcium

Water-soluble calcium tests indicated a high differential between soils, in two portions of the same nursery bed, which proved toxic and non-toxic to young seedlings developed from the same lot of seed (figs. 4 and 5). In the portion of the bed which produced thrifty seedlings (fig. 5), the surface inch of soil, with a pH value of 4.5, contained from 40 to 50 p.p.m. of water-

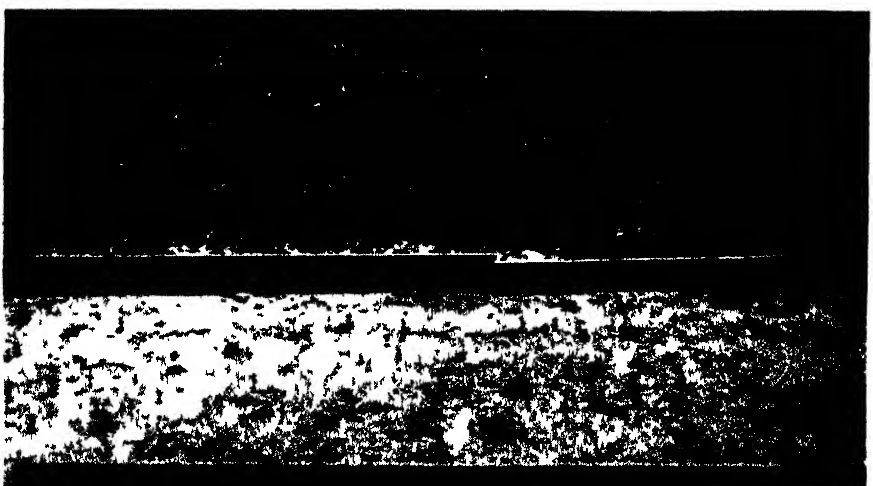


Fig. 4. Portion of nursery bed having 500 p.p.m. of water-soluble calcium and a pH of 7.8, in which shortleaf pine seedlings failed. In background, on similar soil, heavy growth of red clover.

FIG. 5. Portion of same bed illustrated in figure 4 having 40 p.p.m. of water-soluble calcium and a pH of 5.4, in which shortleaf pine seedlings survived and developed well. In background, on similar soil, alsike clover.

soluble calcium. In a portion of the bed where more than 98 per cent. of the seedlings died soon after emergence (fig. 4), the surface inch of soil, with a pH value of 7.8, contained 500 p.p.m. of water-soluble calcium. A thicker, harder crust formed on the surface of the alkaline soil than on that of the acid soil. This crust was high in calcium; probably evaporation had effected a concentration of $Ca(HCO_3)_2$.

Determinations of the pH values of the soil in different portions of this nursery bed were confirmed by the behavior of red clover, *Trifolium pratense* L., and alsike clover, *T. hybridum* L., sown in mixture in an adjoining bed as a soiling crop. It is known that red clover develops well only on neutral or alkaline soils, and that alsike clover develops well on acid soils. On soil adjacent to that determined to have a pH of 7.8, a dense cover of red clover with little or no alsike developed (fig. 4); on soil adjacent to that determined to have a pH of 4.5, a less dense stand of alsike clover (fig. 5).

Discussion

The results of the present investigations contribute to isolation of the factors which confine the germination and survival of shortleaf pine to acid soil media low in soluble calcium. High soluble-calcium content and low hydrogen-ion concentration, or either one, may be lethal to germinating seed or young seedlings of this species (figs. 1 and 4, and table III). The effects of the factors studied are certainly direct physiological ones, not indirect ones brought about through creation of substratum conditions more favorable to damping-off fungi, since examination of the dying seedlings indicated no attack by parasitic fungi. This explanation is counter to that offered by ILLICK and AUGANBAUGH (5) for nonoccurrence of pitch pine on calcareous soils.

It appears improbable, because of the osmotic-pressure values determined (table IV) and of absence of flaccid cells from root sections submersed in culture solutions, that the injury took the form of plasmolysis of the root cells. This is particularly true for seedlings grown in nursery soil having an osmotic pressure of only 0.42 atmosphere. It is recognized that the osmotic pressure of cell sap may be affected by such factors as water content of cell and soluble organic and inorganic substances in the cell, which in turn vary with conditions of the substratum and with growth processes. The value (5.30) for the expressed root sap at the time of determination was not static, but subject to change within certain limits in the direction of change of solute content of the culture medium. MEYER and ANDERSON (6) recognized that "all species can become adjusted within limits to a change in the mineral salt content of the substratum. This adjustment takes the form of an increase in the osmotic pressure of the plant with an increase in the osmotic pressure of the medium from which it obtains its mineral salts." In view of such response of plants to osmotic-pressure changes in their culture media, it seems that shortleaf pine seedlings were in no danger of plasmolysis or physiological drought in culture solution "k" (table IV).

The pH values of the expressed saps of plants vary according to species, mostly between 3.0 and 7.0 according to MEYER and ANDERSON and others. For a particular species, the value may vary—within rather definite limits—from time to time and from one part of a plant to another without injurious effects. TRUOG and MEACHAM (9) and HAAS (2) found that the juices of most plants grown in unlimed soils were more acid than the juices of plants grown in limed soils. While the limits within which the pH values of sap of shortleaf pine seedling roots may fluctuate without injury have not been determined, it has been established that the values do vary with the acidity of the soil media.

It has been observed (fig. 1, and table III) that seedlings developed at pH 6.23 survive in significantly lower proportion than seedlings developed at lower pH values. Seedlings might be expected to tolerate a somewhat higher pH value in natural soil media than in culture solutions. No effort was made to determine the specific effect or effects of H-ion concentration in the culture solutions upon the cell protoplasm. (See MILLER (7), pp. 45–51, 262–265, and 1087–1088.) Of the many cell structures and processes known to be affected by H^+ and OH^- ions, it is not known which were adversely affected in these experiments when hydroxyl ions became toxic.

While a change in pH may effect a change in the relation of the elements in a soil medium which are essential for metabolic processes in seedling plants, there is little basis for belief that seedlings in the germination stage can be seriously affected by deficiency of minerals in the soil. Small quan-

tities of iron, phosphorus, and other basic ions present in soil media are often rendered unavailable by pH values above 6.5; but materials stored in the cotyledons are sufficient to support growth beyond the period in which most of the seedling deaths in these experiments took place.

The cytoplasm and cell sap of most plants, known to be buffered differently, are buffered within rather definite limits. It is recognized that the buffering of the expressed sap of shortleaf seedling roots (figs. 2 and 3) in general represents that of the root cells. The curves in figure 2 have two important features. First, both lots of expressed sap were much more highly buffered against the acid than against the alkali. (Cell saps are usually more highly buffered against one than against the other.) The saps were, in fact, buffered against a pH value lower than those found in many natural soils. Second, the sap of the roots from the acid soil was more highly buffered against alkali than the sap of the roots from the alkaline soil. This difference may be attributable to the smaller amount of phosphorus available in the latter soil, evidenced by colorimetric phosphorus tests on the two saps. The curves in figure 3 indicate that buffer capacity was somewhat increased by adding superphosphate to an acid nursery soil in which seedlings were developing; the difference might have been significantly greater if the superphosphate had been applied to an alkaline soil—a point for further study.

On the basis of theory, the explanation may be advanced that the toxic effect of excess calcium on the seedling root cells consists in a change in the permeability of the protoplasmic membranes, the degree of toxicity depending upon the ionic concentration of calcium. Raber (8) and others have expressed the theory that calcium at first decreases the permeability of these membranes and later increases it. In these experiments, increase in permeability may have been sufficient to result in free diffusion of substances in either direction through the cell membranes and finally, in culture media of high soluble-calcium content, in disorganization of the protoplasm in the root cells of seedlings.

The restrictions placed on seedling growth by calcareous soils may have a serious adverse effect on important phases of a forestation program. Shortleaf pine, if planted on old fields with high-calcium soils, may fail to perpetuate itself through natural regeneration. Unless nursery sites are judiciously selected on the basis of species tolerance for soil factors, costly soil-management practices become imperatively necessary for seedling production. As in the nursery beds used in these experiments (figs. 4 and 5), high water-soluble calcium is usually associated with high pH values, and low water-soluble calcium with low pH values. Reliable evidence of the viability of seed of shortleaf pine, necessary to production of high-quality nursery stock, can best be obtained by correlating seed responses with composition and condition of the germination media.

Summary

Germinating seed and young seedlings of shortleaf pine cannot survive in culture media having a soluble-calcium content of approximately 500 p.p.m. or more and a pH value of approximately 6.5 or more or having either of these characteristics. This was evidenced by behavior of seed in greenhouse cultures and of seedlings in nursery beds.

The osmotic pressures of the culture solutions and a solution of the alkaline nursery soil were not sufficiently high to effect plasmolysis of the seedling root cells.

Expressed sap of roots of seedlings a few weeks old was more highly buffered against an acid than against an alkali. Sap from roots developed in an acid soil was more highly buffered against the alkali than was that from roots developed in an alkaline soil. Buffering of the cell sap of roots may be somewhat increased by applying phosphorus to soils low in that element. The pH values of expressed root saps were lower in all instances than those of the soil media in which the roots developed.

Possibilities as to the nature of the toxic effects of high soluble-calcium and low hydrogen-ion concentrations on the root cells are briefly discussed.

High calcium content of the soil may prevent natural reproduction of planted shortleaf pine stands, increase the cost of soil management in forest nurseries, and render inapplicable the results of seed germination tests.

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THE EFFECT OF AERATION ON GROWTH OF THE TOMATO IN NUTRIENT SOLUTION[1]

WILLIAM DONALD DURELL

(WITH NINE FIGURES)

Introduction

It has long been recognized among plant physiologists that the air relations of roots have an extremely important bearing on both the vegetative and the reproductive phases of plant growth. It has been shown many times that on submerged soils and soils impermeable to air, most plants develop poorly or die early. The most common illustration of this fact is the "drowning out" of wheat in the Spring when water collects in small depressions of the ground.

The consensus of opinion among experimenters is that the air relations of roots have a direct influence on the absorption of water and on the absorption of nutrient ions from the soil solution, as well as on the more direct respiratory requirements of the roots as needed for the continual proliferation of new root tissue and root hairs.

Experimentation has shown that it is not the excess of water itself which is injurious, since plants develop perfectly in water cultures. It is, rather, a lack of aeration resulting from root-submersion which is harmful. Many plants grown in a nutrient solution will develop successfully only when the roots have direct access to sufficient oxygen in solution, as when the solution is thoroughly aerated, or when air is carried into the solution by means of continuous solution renewal.

SACHS (**12**), in his early work with nutrient solutions, discovered that the aeration of some of his cultures resulted in increased growth. After his work in 1860, the subject was given attention by various workers from 1901 to date, and it has been repeatedly shown that lack of aeration of the nutrient medium is an extremely important limiting factor in plant growth.

ARKER (**3**), working with lupines, found that root growth was accelerated by passing air through both soil and water cultures. HALL, BRENCHLEY, and UNDERWOOD (**8**), using lupines and barley, found that aeration of the nutrient medium resulted in a 50 per cent. increase in total dry weight of plants. PEMBER (**11**), found that barley plants did not respond to aeration when grown in solutions renewed periodically every two weeks. FREE (**7**), working with buckwheat in solutions which were renewed every two weeks found that bubbling air, oxygen, or nitrogen through the culture solutions produced neither beneficial nor injurious effects, but that the same treatment with carbon dioxide caused injury within a few hours, and death after a few days.

[1] This investigation was supported in part by a research grant from the Research Committee of the Graduate School, University of Kansas.

Allison (1) and Allison and Shive (2), showed that continuously renewed solutions produced soybean plants which were superior in all respects to those grown in periodically renewed solutions. Aeration of periodically renewed solutions resulted only in an increased root development. Aeration of continuously renewed solutions resulted in a marked increase of both tops and roots.

Knight (9), using maize plants in soil cultures, found that aeration of the roots brought about a definite increase in dry weight. In water cultures, the maize plants failed to respond to aeration. It is noted here, however, that *Elodea canadensis* was used to aerate the solution, and that the quantity of oxygen liberated into the solution by this method may well have been too little to affect the growth of maize. Wallflowers and *Chenopodium album* on the other hand, showed considerable increase in dry weight when aerated by this method. Knight also found that the root growth of maize was correlated inversely with the carbon dioxide content of the solution, rather than directly with the oxygen content.

Clark and Shive (5), showed that aeration of continuously renewed solutions produced a marked increase in growth of both tops and roots of the tomato. The influence of aeration upon top growth was more pronounced than it was upon root growth. Although the plants in the non-aerated solution were much smaller, they started to blossom and fruit earlier than did those in aerated cultures. At the time of harvest (81 days) the aerated plants, however, showed evidence of yielding a much larger crop of fruit than the non-aerated plants.

Loehwing (10), working with the sunflower and soybean in soil and sand cultures, found that aeration, providing less than 10 liters of air per kilogram of soil or sand per day caused early rapid growth and produced taller and heavier plants; it also resulted in larger root systems, more rapid nutrient absorption, and a much increased total weight. When more than 10 liters of air per kilogram of soil or sand was used, however, the plants were injured and retarded to a point even below that of the controls. This work suggests the possibility, under certain conditions, of reaching a point of excessive aeration which might have an adverse effect on plant growth. There is a possibility that Loehwing's results might not apply to the aeration of a nutrient solution, where there would be no possibility of mechanical drying out of the roots.

Arrington and Shive (4), using the tomato, showed that aeration of a continuously renewed nutrient solution produced a marked increase in the absorption rates of cation, anion, and total nitrogen over the corresponding rates from a non-aerated solution. Yields produced by aerated cultures were approximately double the yields produced by the non-aerated cultures. Carbon dioxide accumulation in the culture solutions was found to be with-

out effect on growth, rate of nitrogen absorption, or oxygen content of the solutions. This work of ARRINGTON and SHIVE demonstrated that lack of dissolved oxygen in the culture solution is a limiting factor in the growth of the tomato, rather than the carbon dioxide content, as suggested by FREE (7), and by KNIGHT (9).

It will be seen from this review of experiments on lupines, barley, buckwheat, soybeans, and tomatoes, that there is much evidence to indicate that aeration of the nutrient solution produces plants superior in vegetative growth to those grown in unaerated solutions. It is also apparent that past investigators have used a number of widely different methods for supplying the nutrient solution to the plant. Some of these methods have undoubtedly resulted in efficient aeration of the solution; others have resulted in varying degrees of insufficient aeration. These various methods might be outlined as follows:

1. Unaerated, unrenewed solutions, where the plant is allowed to complete its growth without the solution's being changed, renewed, agitated, or aerated.

2. Unaerated solutions replaced periodically by removing all the old solution and refilling to volume with new.

3. Unaerated solutions renewed periodically by adding new solution to volume.

4. Unaerated solutions renewed continuously with fresh solution, added by means of a drip.

5. Unaerated solutions renewed periodically by analysis and replacement of water and absorbed salts.

6. Unrenewed solutions aerated by bubbling air through the solution.

7. Periodically renewed solutions aerated by bubbling air through the solution.

8. Continuously renewed solutions aerated by air which is carried in along with the new solution.

9. Solutions periodically renewed but continuously circulated, and aerated by air which is forced in by the circulation mechanism.

As far as is known, no quantitative study has been made concerning the effects on plant growth of different degrees of aeration of the nutrient solution; nor has any optimum point been found, with regard to degree of aeration, for either vegetative growth or fruit production. As stated by ALLISON and SHIVE (2), it is impossible, on the basis of our present knowledge, to specify optimum conditions as regards oxygen requirements for plants in general, since these requirements have been shown to be distinctly variable among different species and even different varieties. It should be entirely possible, however, to specify through experimentation. the optimum conditions regarding oxygen requirements for a particular variety of plant in a given nutrient medium under controlled conditions.

It is evident that there is need for more knowledge concerning the effects of aeration on plant growth. Until we can discover the optimum air requirements of roots for certain plants under controlled conditions, and until we can standardize our treatment of such plants, our conclusions in the field of plant nutrition must necessarily be incomplete.

In view of the dearth of experimental information regarding quantitative aeration requirements of plants such as the tomato, and in view of the many existing commercial greenhouse installations for the use of nutrient solution cultures in producing crops of various plants, and of the distinct future commercial possibilities in this direction, it seemed advisable to set up an experiment with the following aims in view:

1. To determine, if possible, the optimum aeration for both vegetative and reproductive growth of the tomato plant.

2. To determine, if possible, the effect of varying amounts of aeration on total fruit production and on speed of fruit production as well as on dry weight of leaves, stems, and roots.

Procedure

The plant of the experiment involved a study of the tomato plant, variety Louisiana Red, as grown in nutrient solution, receiving five different treatments as regards aeration of the roots.

Seeds were planted in flats of clean sand, and the seedlings grown there for approximately three weeks, receiving frequent watering with the same nutrient solution that was used later in the experiment. When about 7 cm. high, the young plants were transferred to their permanent locations in the excelsior screens.

The nutrient solution used for all cultures was that found by SHIVE and ROBBINS (13), to produce excellent growth of tomatoes under average greenhouse conditions. It was composed as follows:

Salts	KH_2PO_4	$Ca(NO_3)_2 \cdot 4H_2O$	$MgSO_4 \cdot 7H_2O$	$(NH_4)_2SO_4$
Molar concentration	0.0023	0.0045	0.0023	0.0007

In order to supply the necessary trace elements, a supplementary solution which had proved beneficial in previous experiments at the University of Kansas was added to the nutrient solution in the following amounts:

Salts	H_3BO_3	$MnSO_4 \cdot 7H_2O$	$ZnSO_4 \cdot 7H_2O$	$CuSO_4 \cdot 5H_2O$
Parts per million	0.5	0.5	0.2	0.1

As a source of iron, iron tartrate in 0.5 per cent. solution was added regularly to the nutrient solution to give a concentration approximating 0.5 parts per million. Throughout the experiment, chlorosis was entirely prevented by the addition of iron tartrate in combination with carefully con-

trolled acidity of the solution. Daily titrations were made, and the hydrogen-ion concentration of the solutions was carefully kept within a range of pH 5.0 to 6.0, which was shown by CLARK and SHIVE (**6**), to include the point of maximum nitrate nitrogen absorption.

The culture tanks (4 feet by 10 feet by 6 inches) were made of heavy-gauge black iron, welded, and coated on the inside with hot asphalt.

Four snugly-fitting frames were constructed out of 2- by 4-inch lumber for each tank. One-inch-mesh iron wire screening was attached to the bottom of each frame, and the whole unit coated heavily with hot asphalt. Clean white pine excelsior was then placed in each screen to a depth of four inches to form a permeable supporting medium for the plants.

Asphalt covered heating cable was installed in the bottom of each tank and the entire installation regulated thermostatically to keep the solutions at a temperature ranging between 75° and 80° F.

Seedlings selected for uniformity were placed in the screens, sixteen to a tank, so that their roots were immersed in the solution which was two inches below the screen.

Tank no. 1 was filled with a mixture of one-third well-rotted cow manure and two-thirds sandy loam, as is practised in the commercial growing of tomatoes. This tank was furnished with several drainage outlets at the bottom, had no excelsior screen, and was watered regularly with water only. This tank was instituted here to compare the results of normal plant growth in soil with the others of the series.

Tank no. 2, in addition to the excelsior screen covering the solution, was fitted with a layer of heavy asphalt and sisal-bonded paper so that the plant stems projected through small holes. This was arranged in order to prevent, as much as possible, any diffusion of air into the solution. This treatment was instituted as a control to determine the results of growth in a solution lacking aeration.

All other treatments were set up to furnish varying degrees of aeration of the solution and to determine its effect on growth. In tank no. 3, as in nos. 4, 5, and 6, the excelsior screen was left uncovered and open to maximum diffusion of air through the screen into the solution.

Tank no. 4 was set up with a continuous drip-bubble apparatus after the method of SHIVE and STAHL (**14**). A 50-gallon accessory tank of nutrient solution was connected with a length of rubber tubing, and the solution fed into a Pyrex capillary feed-tube drop by drop, each drop carrying with it into the solution a definite amount of trapped air. At no time was there any overflow from this tank. A series of pinch-clamps regulated the flow of solution, and only enough was supplied to keep the level of the solution at a point corresponding with that of all other tanks. As the plants in this tank matured, it was necessary to increase the rate of flow of solution, and

consequently, the amount of air supplied to the solution. Weekly checks were made, however, on the amount of air supplied, and the calculation of 2.5 ml. of air per plant per minute for tank no. 4 is based on the average of these observations.

Tank no. 5 was furnished with a supply of compressed air giving 37.5 ml. of air per plant per minute. The air was supplied at this rate continuously, and was broken up by means of an aspirator into extremely small bubbles when making contact with the solution.

In tank no. 6, the solution level was slightly lower than in the others. The excess solution was led by gravity through an overflow pipe into a small covered tank. As the solution accumulated in this small tank, it actuated a centrifugal pump, thus pumping the excess solution into an overhead tank. From this tank, the solution flowed by gravity again into the main no. 6 tank, providing a continual circulation of the solution. The overhead tank supplied enough pressure that the solution could be squirted through Pyrex nozzles with some force into the main no. 6 tank in which the plants were growing. The average amount of air delivered into the solution, along with the returning solution, was calculated to be 250 ml. per plant per minute. The amounts of air used here were not planned as ideal quantities, but were rather the uncalculated results of efficiently functioning equipment. While not ideal for their purpose, it was felt that they would give useful indications of the effects of greatly differing amounts of aeration.

It should be noted here that the plants in all tanks but no. 1 received similar treatment except as to aeration of the nutrient medium. Daytime air temperature was kept between 65° and 70° F. and at night between 60° and 65° F. The temperature (75°–80° F.) of the nutrient medium was in all cases the same. All plants received the same solution and all received the same amount of new solution per plant per day, as well as the same amount of total solution per plant.

Tanks no. 2, 3, 5, and 6, were given a supply of new solution to volume every 48 hours. Tank no. 4 differed only in that it received its supply of new solution to volume continuously, drop by drop.

For purposes of clarity in discussing experimental results, use will be made of the following descriptive terms: Treatment no. 1 will designate treatment of plants growing in tank no. 1, treatment no. 2 will designate treatment of plants growing in tank no. 2, etc.

Results

The cultures described here were started on January 1, 1939, and all plants were harvested on May 21, 1939. Fruit was picked and weighed as it ripened, and an accurate account was kept of the daily production of plants receiving different treatments. Leaves and petioles fallen by abscis-

sion were kept at the base of each plant for inclusion in the harvest. The dry weights quoted were obtained by storing the material in a hot dry room for several months, then drying it further in a vacuum oven at 80°.

Fruit production

The average fruit production per plant for each treatment based on the unit of aeration is shown in figure 1.

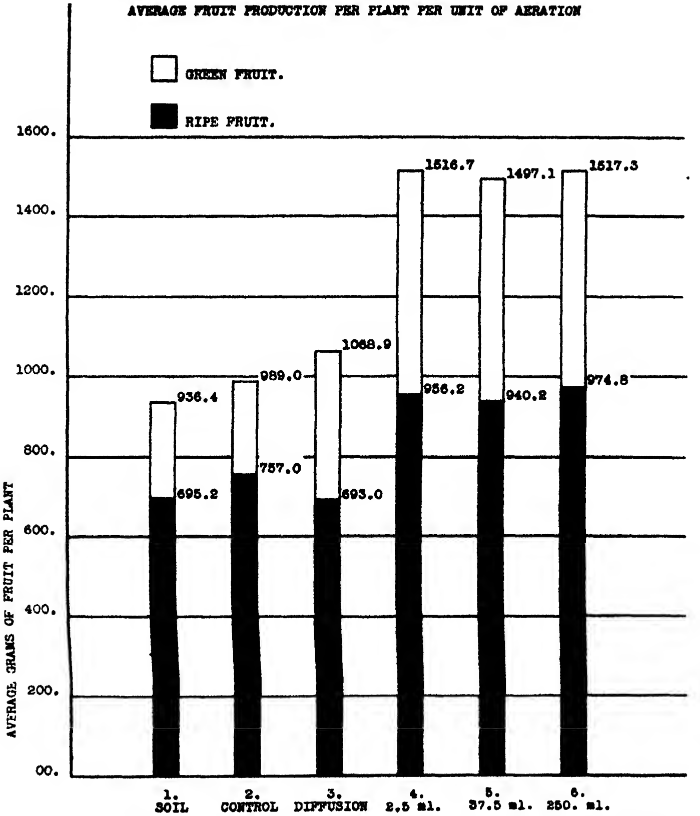

Fig. 1. Average fruit production per plant per unit of aeration.

The increase in both ripe fruit and total fruit production, due to aeration of the nutrient solution, is clearly illustrated by this table. The difference in results between treatments 3 and 4 is highly significant. The differences, however, between treatments 1, 2, and 3, and the differences between treatments 4, 5, and 6, are not considered significant, as the percentage variation is less than 10 per cent. The average speed of ripe fruit production per plant for each treatment, based on the number of days of growth, is shown in figure 2.

An interpretation of these results indicates that an extremely small amount of aeration of the nutrient medium, such as that of treatment no. 3, has a beneficial effect on the speed of ripe fruit production only in the early stages of growth. Plants receiving treatment no. 3, however, show a distinct lag in speed of ripe fruit production, especially in the later period of growth, behind plants given treatments no. 4, 5, and 6, all of which received a greater amount of air than those given treatment no. 3.

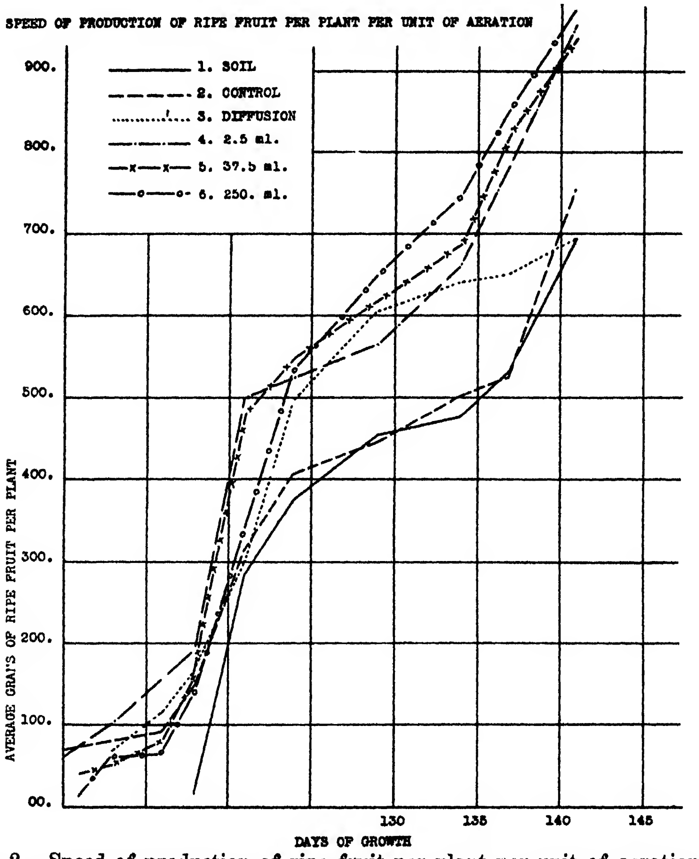

FIG. 2. Speed of production of ripe fruit per plant per unit of aeration.

On the other hand, a greater degree of aeration, as represented by treatments no. 4, 5, and 6, had a much more beneficial effect on both total fruit production and speed of ripe fruit production.

The most pronounced feature of these results, however, is the fact that the aeration requirements for optimum fruit production are low. Amounts of air greater than 2.5 ml. per plant per minute have very little effect on either total fruit production or speed of ripe fruit production. This can

perhaps best be noted in figure 3, where the results of treatments 2, 4, 5, and 6, supplying no air, 2.5 ml., 37.5 ml., and 250.0 ml. of air, respectively, per plant per minute are plotted in a curve based on amount of air per plant per minute.

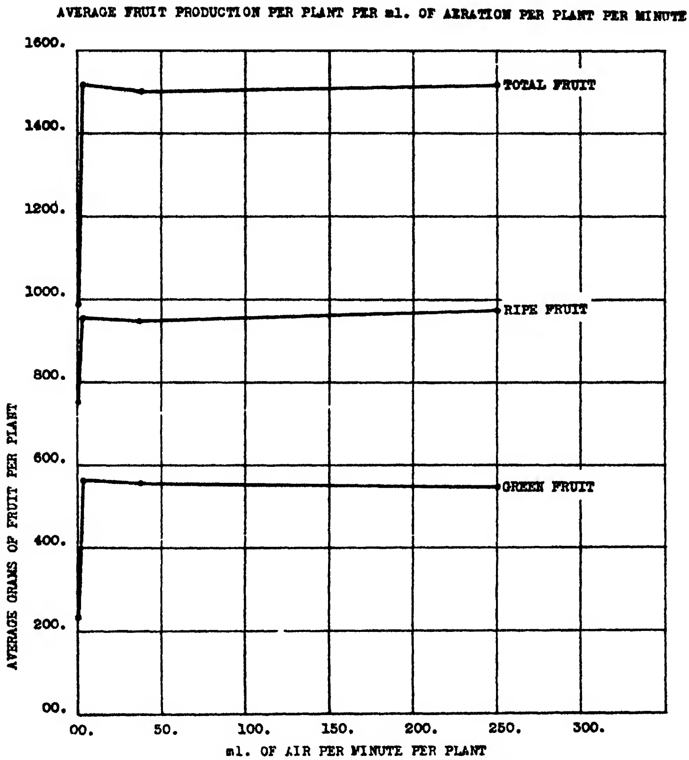

FIG. 3. Average fruit production per plant per ml. of aeration per plant per minute.

The slight superiority of treatment no. 2 over the soil grown plants of no. 1 is not significant; it might possibly be due to a stimulus of the reproductive phase of the plant, brought about by a lack of oxygen. This conclusion is apparently borne out by the fact that treatment no. 2 produced less vegetative growth than any one of the other treatments including no. 1.

Vegetative growth

The average dry weight of roots per plant, based on the unit of aeration is illustrated in figure 4.

The beneficial effect of air is clearly shown here by the fact that treatment no. 2, with no aeration, produced the smallest dry weight of roots. Treatments no. 1 (the soil grown plants) and no. 3 (receiving diffused air) each produced significantly larger quantities of roots than did treatment

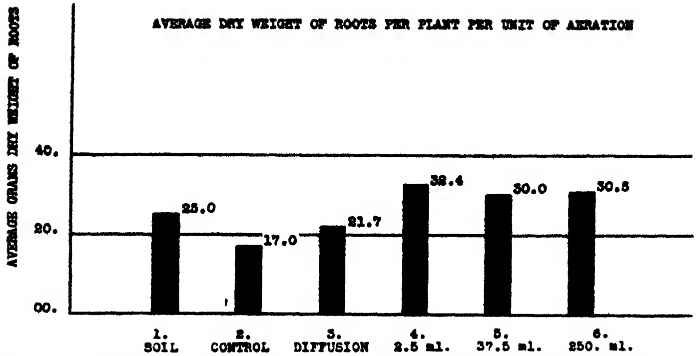

FIG. 4. Average dry weight of roots per plant per unit of aeration.

no. 2; treatments 4, 5, and 6, all receiving an aeration of more than 2.5 ml. per plant per minute each produced much larger quantities of roots than either no. 1, 2, or 3. These results indicate that, as in the case of fruit

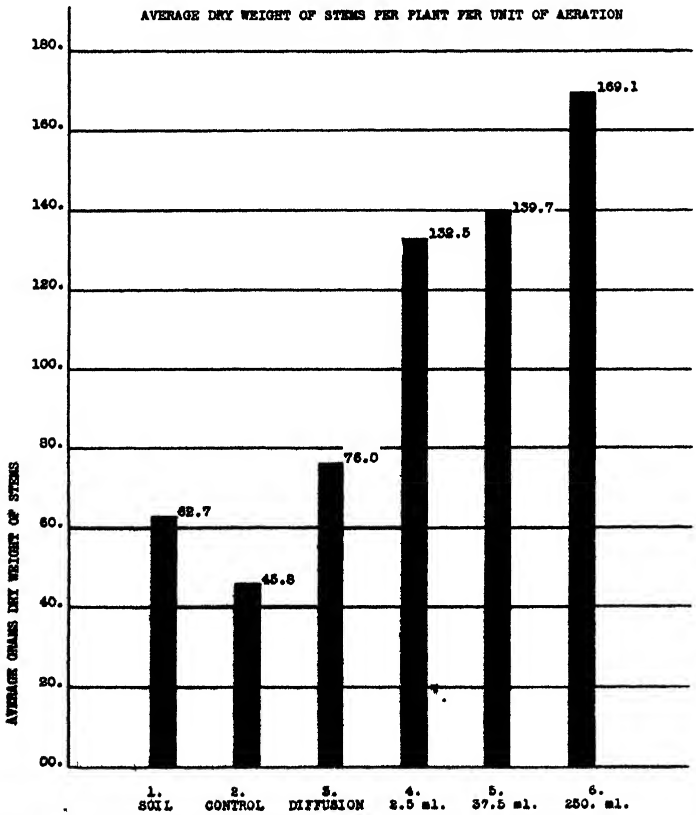

FIG. 5. Average dry weight of stems per plant per unit of aeration.

production, the air requirement for optimum root production is low; amounts of air over 2.5 ml. per plant per minute have very little effect on the production of roots.

Figures 5 and 6 show the average dry weight of stems and leaves per

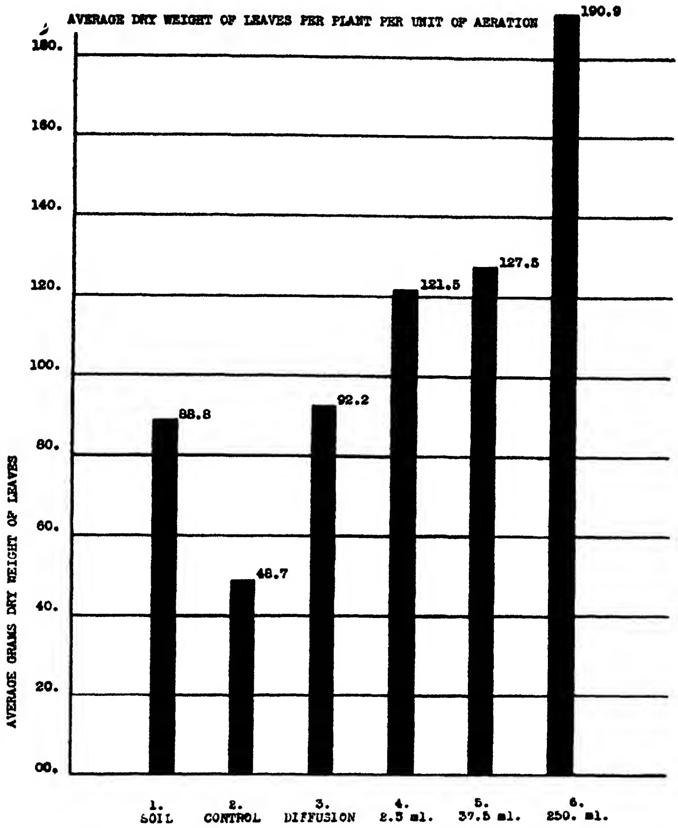

FIG. 6. Average dry weight of leaves per plant per unit of aeration.

plant, based on the unit of aeration. The beneficial effect of aeration is clearly shown in both tables. The smallest average weight of both stems and leaves was produced by plants receiving treatment no. 2, which provided no air, while the dry weights of plants receiving the other treatments are seen to exhibit a proportional response to the amount of aeration per plant per minute.

An interpretation of the results indicates that the aeration requirements for optimum stem and leaf production are high. This is shown by the fact that the greatest amount of both stems and leaves was produced by plants receiving treatment no. 6, which provided the greatest amount of air. It is

possible that the optimum amount of aeration for stem and leaf production was not reached in these experiments.

The average, total dry weight per plant including leaves, stems, and roots, based on the unit of aeration is illustrated in figure 7.

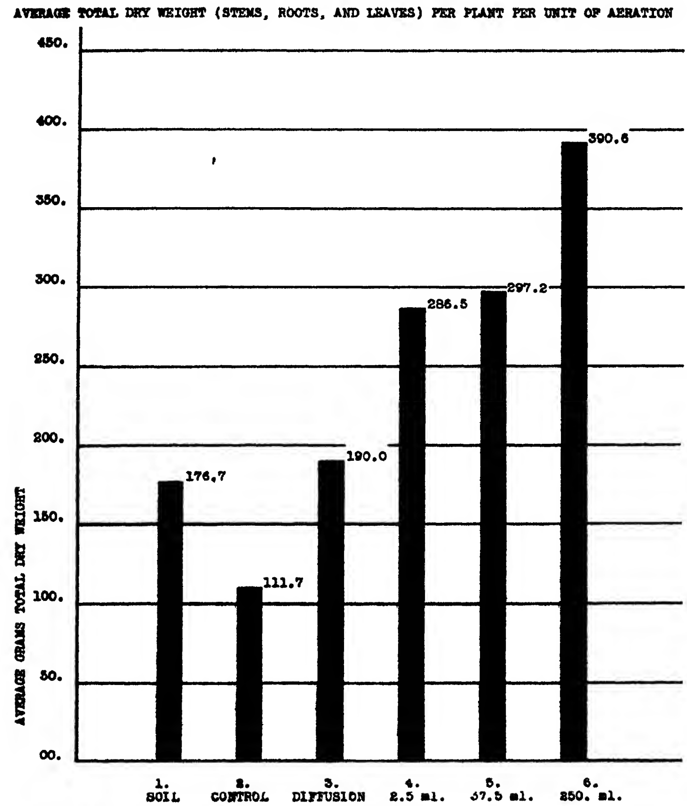

FIG. 7. Average total dry weight (stems, roots, and leaves) per plant per unit of aeration.

Figure 8 shows the effect of increasing amounts of aeration on total dry weight. The rising curve here again indicates, perhaps more clearly, the possibility that the optimum amount of aeration for total vegetative growth was not reached in these experiments. On the other hand, the marked increase in dry weight due to even a small amount of aeration is clearly shown.

No attempt was made to determine the various oxygen tensions of the solutions receiving different air treatments. As shown by figures 6, 7, and 8, however, it is possible that none of the cultures received the amount of dissolved oxygen in the solution which was necessary for optimum vegetative growth. The possibility is clear therefore, that none of the culture solutions was aerated to a point of oxygen saturation.

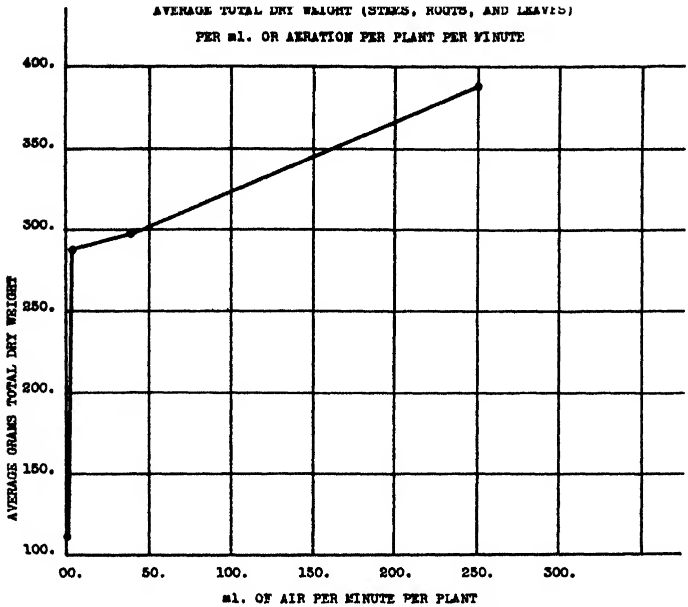

FIG. 8. Average total dry weight (stems, roots, and leaves) per ml. of aeration per plant per minute.

Several interesting observations were made during the course of the experiment.

The roots growing in tank no. 2, unaerated, tended to project their root-tips above the level of the solution, and to produce a large number of long-

FIG. 9. Root system of mature plants grown in tank no. 2. Several root tips shown projecting above the surface of the solution.

lived root hairs in the damp atmosphere between the solution and the asphalt-paper covering. The roots of the aerated tanks, however, kept their root-tips below the level of the solution and produced a very small number of root-hairs.

The fruit produced by these plants was sampled by several persons, and no noticeable effect of aeration on the quality of the fruit was discovered. The fruit produced by solution-culture plants, however, was definitely firmer and meatier than that produced by the soil-grown plants. This observation was confirmed when some of the fruit was canned.

At the time the plants were harvested, plants receiving treatments number 1, 2, and 3, had slowed down considerably in their rate of vegetative growth; plants in tanks 4, 5, and 6, however, appeared to be continuing their vegetative growth at an unchanged rate.

Summary

In this paper a quantitative study was made of the effects of aeration of the nutrient solution, as related to the fruit production and vegetative growth of the tomato.

1. Aeration was shown to have a decidedly beneficial effect on the production of fruit, as well as on the vegetative growth of roots, stems, and leaves.

2. Aeration of the nutrient medium merely by natural diffusion of air had no significant effect on total fruit production or upon the speed of ripe fruit production, except in the early stages of growth.

3. Aeration of the nutrient medium by artificially supplying the solution with 2.5 ml. of air per plant per minute, or more, greatly increased both total fruit production and the speed of ripe fruit production.

4. Optimum fruit production was obtained when the nutrient solution was supplied with 2.5 ml. of air per plant per minute. Increasing the rate of aeration was without effect.

5. Optimum production of roots was obtained when the nutrient solution was supplied with 2.5 ml. of air per plant per minute. Increasing the rate of aeration was without effect.

6. Optimum production of stems and leaves was probably not obtained in this experiment. Stem and leaf production, within the limits of the experiment, are shown to be proportional to the rate of aeration; the greatest production was obtained with a supply of 250 ml. of air per plant per minute.

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LACTIC ACID FORMATION IN ALCOHOLIC FERMENTATION BY YEAST

LEONORA A. HOHL AND M. A. JOSLYN

(WITH FOUR FIGURES)

Introduction

The rôle of lactic acid in alcoholic fermentation has been of interest to all investigators who have concerned themselves with the chemistry of this process. PASTEUR (**25**) did not regard lactic acid as a product of normal alcoholic fermentations. His evidence indicated that its occurrence in fermented media was always associated with the presence of contaminating organisms. Subsequently, its occurrence in alcoholic fermentations, free of bacteria, was established, and several theories were proposed in which lactic acid played the rôle of a three-carbon intermediate between sugar and the end products, carbon dioxide and alcohol. BUCHNER and MEISENHEIMER (**3**) on the basis of their work with yeast press juice, first reported lactic acid to be such an intermediate; later, however, as a result of experiments made with pure yeast cultures, they concluded that live yeasts neither form nor ferment lactic acid. SLATOR (**29**) failed to agree with BUCHNER and MEISENHEIMER's early work, considering lactic acid as a by-product rather than intermediate in alcoholic fermentation. The proponents of the WOHL-SCHADE (**6, 18, 26**) hypothesis held lactic acid to be an intermediate product in alcoholic fermentation. NEUBERG and TIR (**23**), FERNBACH and SCHOEN (**9, 10**), and EULER and LINDNER (**8**), also presented evidence for the intermediate rôle of lactic acid in alcoholic fermentation. Among those who could not confirm this evidence were KERB and ZECKENDORF (**16**), and KOSTYCHEV and FREY (**17**). OPPENHEIMER (**24**) considered lactic acid to be a by-product of alcoholic fermentation. His work indicates that either dihydroxyacetone or glyceric aldehyde (probably the latter), may be regarded as precursors of lactic acid. Meanwhile NEUBERG (**20, 21, 22**), presented evidence to show that methyl glyoxal was the precursor of lactic acid in both yeast fermentation and that brought about by animal tissues. He considered the formation of lactic acid from sugar quite probable. The foregoing as well as many other investigations (**27, 28**), of the rôle of lactic acid in alcoholic fermentation were conducted chiefly to determine the mechanism of its formation and cannot be cited as evidence of its occurrence as a by-product of normal alcoholic fermentation. The frequent occurrence of lactic acid bacteria in alcoholic fermentations makes many of the early investigations of doubtful value. Recent reports (**1, 5, 7, 13**) have postulated the formation of lactic acid as a by-product of alcoholic fermentation of sugar by pure yeasts. DURMISHIDZE (**5**) concludes that lactic acid is a

true by-product of alcoholic fermentation of sugars and is not formed from malic acid nor in significant amounts from the nitrogenous constituents of the medium. It is a constant by-product and formed to about the same extent by several strains of yeast examined.

The purpose of this investigation is to determine the course of lactic acid formation in relation to the utilization of sugar during alcoholic fermentation by pure yeasts and to determine the effect of some external conditions upon the total quantities of lactic acid formed by these yeasts.

Methods

Seven closely related strains of yeast were used in this investigation. They were all found to be strains of *Saccharomyces cereviseae* (HANSEN) STELLING-DEKKER, when classified according to STELLING-DEKKER (**30**). The strains designated as Burgundy and Champagne were originally from the collection of PACOTTET of France (**2**). Numbers 2338 and 2368 were obtained from the American Type Culture Collection in July, 1937. The former was labelled *Saccharomyces ellipsoideus,* and the latter *Saccharomyces cereviseae.* The Tokay strain was originally from California Fruit Industries, Ltd. Yeast number 66 was isolated by CRUESS (**4**) and the FREI BROTHERS' (FB) strain was isolated by the writers from a naturally fermenting vat of wine at Frei Brothers' Winery, Healdsburg, California, 1935 vintage.

The media used for the experiments included grape juice, wort, and an artificial medium which was a modification of WILLIAMS and SAUNDERS' (**31**) medium with the rare elements and asparagine replaced by a small amount of yeast extract. The composition of this artificial medium was 200 gm. dextrose, 3 gm. ammonium sulphate, 2 gm. potassium acid phosphate, 0.25 gm. calcium chloride ($CaCl_2 \cdot 2H_2O$), 0.25 gm. magnesium sulphate, and 30 ml. yeast extract (containing 0.25 per cent. nitrogen, and 1.5 per cent. total solids), per liter. The media were sterilized in flowing steam for a period of one hour on each of three successive days.

Yeast starters were generally prepared by transferring a loopful of yeast from a young stock agar slant to a small volume of the particular medium to be used in the experiment. This was transferred after two or three days to another portion of the same medium. After two more days the experimental media were inoculated, using sterile serological pipettes, with a volume of this second culture, amounting to one per cent. of the volume of the medium being inoculated. The fermentations were conducted at room temperature, 18–25° C., averaging 22° C. Most of the fermentations were made in duplicate. There was close agreement between duplicates and only the averages are included in the tables.

The total volatile acidity was determined by titrating in the hot, 100 ml.

of steam distillate from a 10-ml. sample. The results thus obtained are probably somewhat high, since variable amounts of lactic acid are volatile with steam. The pH was determined by the quinhydrone electrode. Sugar was determined by HASSID's modification of WHITMOYER's procedure (**14**). Total fixed acidity was determined by subtraction of total volatile acidity from total titratable acidity, the latter being determined by direct titration of a sample diluted with hot water. Lactic acid was determined by the method of FRIEDEMANN and GRAESER (**11**). After evaporating the alcohol, those samples containing less than 0.2 gm. sugar per 100 ml. of medium, were prepared for analysis by treatment with copper sulphate and calcium hydroxide suspension.[1] Aliquot portions of the filtrate were then oxidized and titrated as described by FRIEDEMANN. After removal of alcohol, those samples containing more than 0.2 gm. of sugar per 100 ml., were acidified (with 2 ml. of 1 + 4 H_2SO_4 for each 100 ml. of medium), and extracted with diethyl ether for fifty hours in a continuous liquid extractor. The excess ether was then distilled off, and the sample was made to the original volume in a volumetric flask of suitable size. These extracts were then treated exactly as were the other samples. This was necessary to avoid errors due to aldehyde formation on oxidation of malic, tartaric, and succinic acids (**11**).

Results and discussion

LACTIC ACID FORMATION DURING FERMENTATION OF GRAPE MEDIUM

Tall eight-liter bottles fitted with sampling tubes, were filled with six liters of Thompson Seedless grape juice, having 116 milli-mols (20.9 gm.) of total reducing sugar (as dextrose), 6.34 milli-equivalents of total acid, 3.73 milli-equivalents of tartrate, and 0.12 milli-equivalents of total volatile acid per 100 ml. The pH was 3.67. This medium did not contain any lactic acid but had a trace of formic acid. These were sterilized and individual bottles inoculated with the seven pure yeast strains. Samples of approximately 100 ml. were withdrawn from the fermentations at intervals determined by the relative rate of fermentation over a total period of 75 days. The yeasts were removed from the samples by centrifuging, and the samples were then frozen and stored at $-18°$ C. until the analyses could be made.[2] At the end of the experiment the yeast sediment was examined microscopically and both dilution and streak plates were made on suitable media to verify the purity of the cultures. No contaminants were found.

The data obtained for Frei Brothers' yeast are given in table I, and

[1] Preliminary experiments on known solutions showed that there was 95 per cent. recovery of lactic acid from samples thus treated.

[2] Before the total titratable acidity was determined the tartrates which had been precipitated as a result of freezing were brought back into solution by heating the samples in crown capped bottles and allowing them to cool again to room temperature.

TABLE I

FORMATION OF LACTIC ACID DURING ALCOHOLIC FERMENTATION OF GRAPE MEDIUM BY FREI BROTHERS' YEAST

TIME	SUGAR UTILIZED*	TOTAL VOLATILE ACID FORMED†	TOTAL FIXED ACID FORMED†	LACTIC ACID FORMED	RATIO EQUIVALENT LACTIC ACID / EQUIVALENT VOLATILE ACID
	m. mols	m. eq.	m. eq.	m. eq.	
12 hr.	7	0.04	0.10	0.11	2.75
24 "	12	0.37	0.49		
36 "	20	0.49	0.87	0.29	0.59
48 "	24	0.50	1.12	0.25	0.50
59 "	31	0.53	1.23		
72 "	50	0.71	1.17		
83 "	67	0.72	1.25	0.35	0.48
96 "	67	0.73	1.32	0.37	0.50
108 "	72	0.75	1.25		
120 "	82	0.79	1.39	0.49	0.62
6 days	88	0.81	1.23	0.62	0.76
7 "	98	0.79	1.07		
8 "	104	0.79	0.93	0.75	0.95
9 "	107	0.83	0.59	0.75	0.90
11 "	111	0.81	0.67	0.89	1.09
14 "	114	0.88	0.91		
17 "	113	0.85	0.56	1.09	1.26
21 "	114	0.86	0.49	1.11	1.40
26 "	114	0.83	0.14	1.31	1.57
31 "	114	0.85	0.21	1.20	1.41
38 "	114	0.86	0.17	1.15	1.33
46 "	114	0.86	−0.04	1.10	1.27
60 "	115	0.86	−0.14	1.17	1.36
75 "	115	0.89	−0.23	1.27	1.42

* Milli-mols per 100 ml.
† Milli-equivalents per 100 ml.

those for yeast 2338 are graphically presented in figure 1. In table II the significant trends in the acid data for all seven strains are summarized. It is evident from these data that lactic acid, in contrast to volatile acid (acetic) was produced throughout the fermentation of the sugar, while volatile acid accumulated most rapidly during the fermentation of the first half of the sugar (15). This fact is reflected in the ratios of the respective acids formed to the sugar utilized. The ratio of volatile acid formed to sugar utilized rose to 0.03 during the first 24 hours of fermentation; during the ensuing 60 hours while the first half of the sugar was being fermented, the ratio dropped to 0.01. It thereafter dropped rather slowly, while the remaining sugar was being fermented, to 0.007, where it remained until the end of the experiment. The ratio of lactic acid formed to sugar utilized, on the other hand, attained its maximum of 0.015 after 24 hours and there-

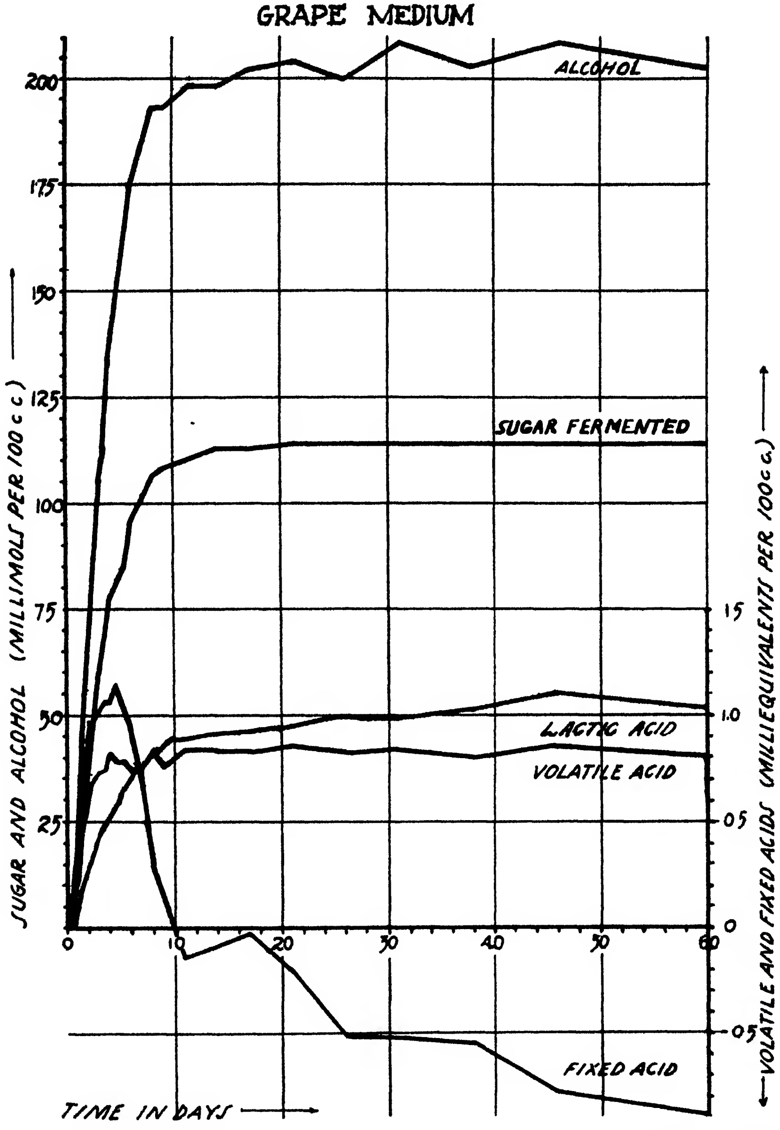

FIG. 1. Formation of lactic acid, volatile acid, and total fixed acid in relation to alcohol formation and sugar utilization in grape medium by yeast 2338.

after remained close to, or slightly below, 0.01. It thus shows a steady formation of lactic acid throughout the course of the fermentation. The same trend is reflected in the ratios of lactic acid to volatile acid formed at the various stages of fermentation.

In figure 2 the relationships between lactic acid formed and sugar uti-

TABLE II

COMPARISON OF ACID FORMATION BY THE SEVEN STRAINS OF YEAST DURING FERMENTATION OF GRAPE MEDIUM*

Yeast	Sugar half fermented			Sugar completely fermented			Final sample		
	Total volatile acid	Total fixed acid	Lactic acid	Total volatile acid	Total fixed acid	Lactic acid	Total volatile acid	Total fixed acid	Lactic acid
	m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*
Burgundy	0.80	0.90	0.44	0.95	−0.07	1.42	0.92	−0.78	1.02
Frei Brothers'	0.71	1.25	0.35	0.83	0.67	0.89	0.89	−0.23	1.27
Tokay	0.81	1.23	0.66	0.84	−0.06	1.20	0.89	−0.87	0.90
2338	0.72	0.98	0.41	0.83	−0.19	0.99	0.84	−0.86	1.19
66	0.63	0.91	0.65	0.74	−0.04	1.07	0.75	−0.99	1.16
Champagne	1.17	0.61	0.66	1.30	−0.16	1.50	1.34	−0.84	1.13
2368	0.68	0.94	0.40	....	....	....	0.87	−1.41	0.89

* Quantities expressed as milli-equivalents per 100 ml.

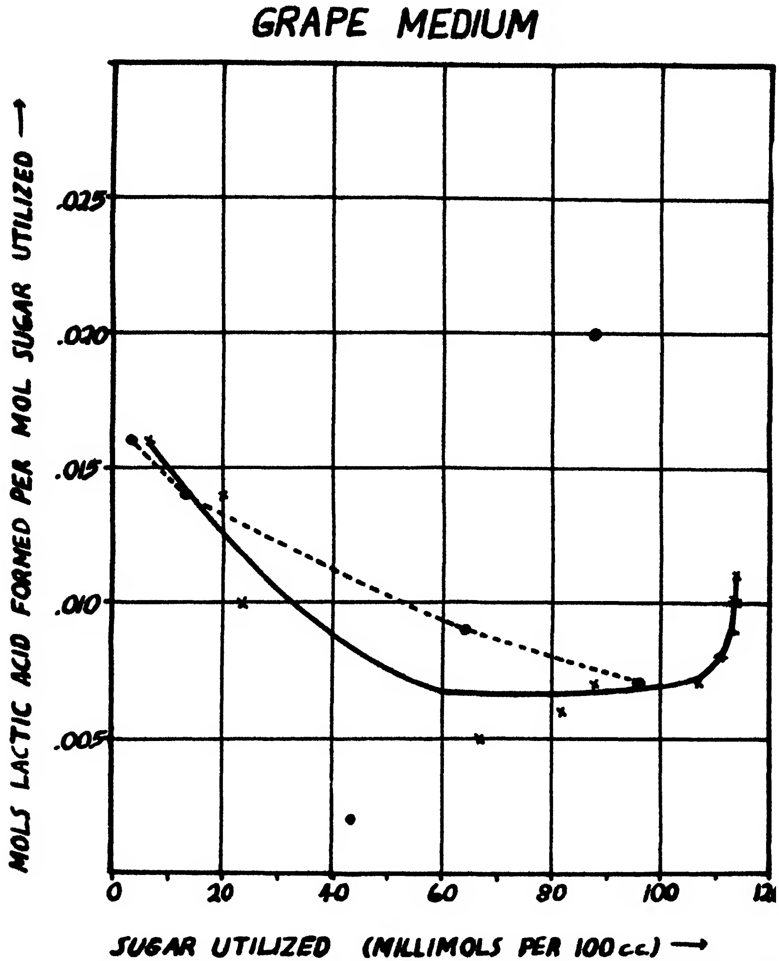

FIG. 2. Relation between lactic acid formed and sugar utilized by Frei Brothers' yeast in grape medium.

X = ratio of total mols lactic acid formed per total mols sugar utilized.

O = differential of increase in mols lactic acid formed to decrease in mols sugar utilized.

lized by Frei Brothers' yeast fermenting the grape medium are graphically presented. The solid line shows the ratio of total lactic acid formed to total sugar utilized, plotted against milli-mols sugar utilized. It may be seen that the ratio was relatively high at the beginning of fermentation and then dropped gently during the main course of the fermentation. During the final stages of the experiment after the sugar had all been utilized, lactic acid continued to be formed, thus producing a steep rise in the curve. This final rise in the curve indicates that in this stage, at least, lactic acid has

some precursor other than sugar; probably organic acid, such as tartaric acid, present in the grape juice medium. The broken line is interpolated among the calculated experimental points representing the ratio of the change in lactic acid to the change in sugar plotted against sugar utilized. This line was carried only to the completion of the sugar utilization since beyond this point it would approach infinity owing to the continued lactic acid formation after complete sugar utilization.

Tables I and II and figure 1 also show that the net total fixed acidity formed during the fermentation of grape juice by these yeasts reached a maximum when approximately half the sugar was fermented, and then dropped to about zero when the sugar was just fermented; it continued to drop thereafter to an average value of about minus one milli-equivalent per 100 ml. of medium at the end of fermentation. Determination of the tar-

TABLE III

FORMATION OF LACTIC ACID DURING ALCOHOLIC FERMENTATION OF ARTIFICIAL MEDIUM BY FREI BROTHERS' YEAST

TIME	SUGAR UTILIZED*	TOTAL VOLATILE ACID FORMED†	TOTAL FIXED ACID FORMED†	LACTIC ACID FORMED†	RATIO EQUIVALENT LACTIC ACID / EQUIVALENT VOLATILE ACID
	m. mols	*m. eq.*	*m. eq.*	*m. eq.*	
24 hr.	5	0.08	0.02	0.05	0.61
48 "	9	0.26	0.10		
60 "	11	0.35	0.21	0.06	0.17
72 "	12	0.43	0.31		
84 "	15	0.48	0.60		
96 "	17	0.55	0.61	0.15	0.27
108 "	18	0.63	0.65		
120 "	20	0.67	0.59		
6 days	26	0.83	0.57	0.18	0.22
7 "	33	1.04	0.64	0.33	0.31
8 "	39	1.12	0.52	0.41	0.36
9 "	47	1.31	0.41		
10 "	51	1.43	0.61		
11 "	58	1.52	0.90	0.52	0.34
12 "	69	1.58	1.08		
13 "	71	1.68	0.94		
14 "	76	1.70	0.92		
16 "	85	1.85	1.55		
18 "	93	1.86	1.76	0.69	0.37
20 "	100	1.98	1.42	0.73	0.37
23 "	102	1.91	1.55	1.12	0.58
27 "	103	2.00	1.86		
32 "	104	1.87	1.69		
38 "	104	1.91	1.63	1.29	0.67
45 "	104	1.96	1.28	1.38	0.70

* Milli-mols per 100 ml.
† Milli-equivalents per 100 ml.

taric acid and tartrates[3] present in the original and final samples showed that this net loss in fixed acid formation was due to utilization of tartrate as an accessory carbon source, which apparently started at an early stage of the fermentation. This utilization of tartrate was sufficient to mask the formation of lactic acid. Frei Brothers' yeast showed the lowest net loss of fixed acid but its lactic acid accumulation was typical of the group.

Table II shows that there were slight individual differences among the yeasts with respect to the quantities and rates of lactic acid formation in the grape medium; in general, however, this acid accumulated throughout the fermentation. With Burgundy, Tokay, and Champagne yeasts there is some evidence of utilization of lactic acid as a carbon source, after sugar had been depleted. MEISSNER (19) observed lactic acid destruction in wines free of sugar to be correlated with an increase in volatile acidity, but he was probably dealing with contaminated cultures. In these experiments there was no marked correlation of this nature.

The pH values remained fairly close to 3.5 throughout fermentation of the grape medium by all seven yeasts.

LACTIC ACID FORMATION DURING FERMENTATION OF ARTIFICIAL MEDIUM

The artificial medium having 106 milli-mols dextrose, 1.77 milli-equivalents total acid per 100 ml., no volatile acid, no lactic acid, and a pH of 4.38, was fermented with the seven yeasts and samples were withdrawn as in the previous experiment. In tables III and IV and in figures 3 and 4 the data are presented in a manner corresponding to that used for the data from the grape medium. In general, the quantities of lactic acid formed and the relation of these to the quantities of sugar utilized by the respective yeasts were very similar in this medium to those observed in the grape medium. The ratios of lactic acid to volatile acid formed were considerably lower in the fermentation of this medium than in that of the grape medium since the actual quantity of volatile acid formed was higher in the artificial medium than in the grape medium. The trend of these ratios, however, was quite comparable in both types of media. From figure 4 it can be seen that, following the initial high point, the ratio of lactic acid formed to sugar utilized remained fairly constant; when the sugar had all been utilized, it rose rapidly because of continued lactic acid formation. The ratio $\frac{\Delta \text{ lactic acid}}{\Delta \text{ sugar}}$ varied in a manner similar to that found in the grape medium. Total fixed acid formation in this medium continued to rise, though irregularly, throughout the entire period of the experiment.

The pH of this medium dropped within the first three or four days from

[3] These were determined by the official procedure of the Association of Official Agricultural Chemists (1935, p. 168.)

TABLE IV

COMPARISON OF ACID FORMATION BY THE SEVEN STRAINS OF YEAST DURING FERMENTATION OF ARTIFICIAL MEDIUM*

YEAST	SUGAR HALF FERMENTED			SUGAR COMPLETELY FERMENTED			FINAL SAMPLE		
	TOTAL VOLATILE ACID	TOTAL FIXED ACID	LACTIC ACID	TOTAL VOLATILE ACID	TOTAL FIXED ACID	LACTIC ACID	TOTAL VOLATILE ACID	TOTAL FIXED ACID	LACTIC ACID
	m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*
Burgundy	1.22	0.88	0.73	1.57	1.51	1.37	1.57	1.83	1.20
Frei Brothers'	1.43	0.61	0.52	1.91	1.55	1.11	1.96	1.28	1.37
Tokay	1.20	0.70	0.71	1.75	1.77	1.21	1.65	1.15	1.15
2338	1.06	1.32	0.52	1.52	1.60	1.19	1.51	2.17	1.25
66	1.13	0.93	0.50	1.40	1.72	1.29	1.40	1.74	1.29
Champagne	1.38	0.86	0.30	1.62	1.56	0.78	1.62	1.70	0.88
2368	1.48	0.66	0.39	......	......	......	1.82	1.30	0.97

* Amounts expressed as milli-equivalents formed per 100 ml.

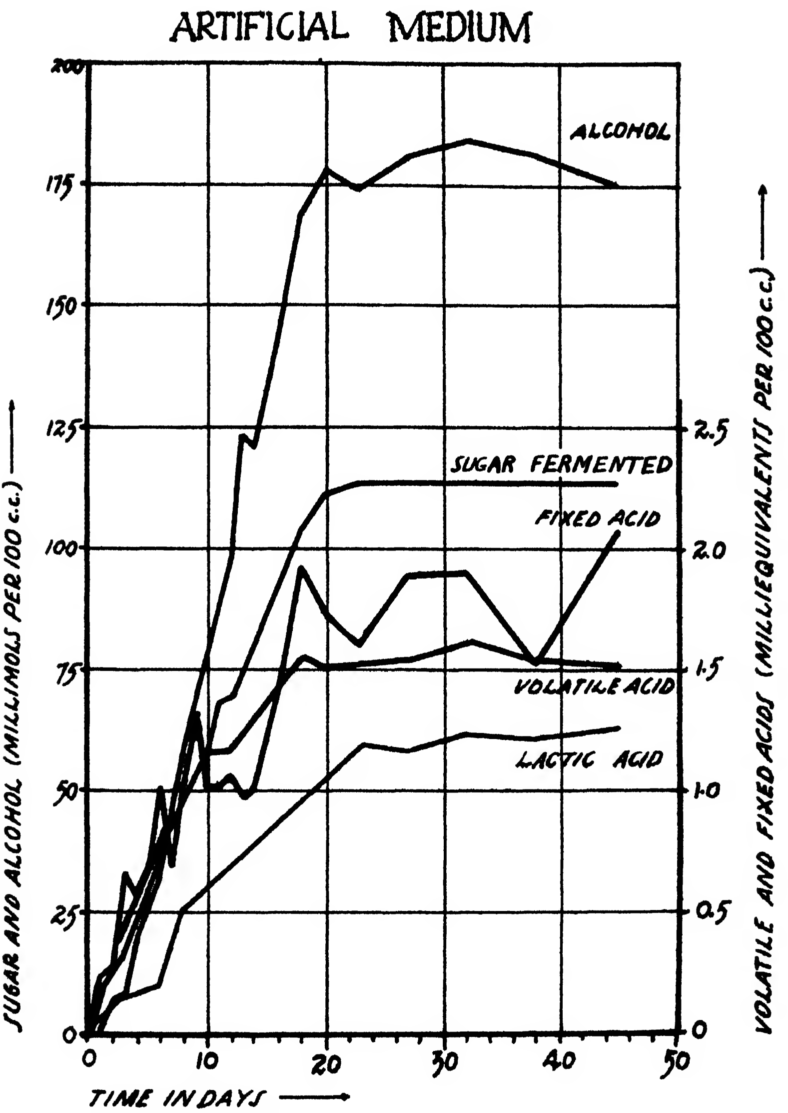

FIG. 3. Formation of lactic acid, volatile acid, and total fixed acid in relation to alcohol formation and sugar utilization in artificial medium by yeast 2338.

its initial value of 4.38 to 2.6–2.8, at which point it remained throughout the fermentation.

No very marked differences were noted among the seven strains with respect to amount and rate of lactic acid formation.

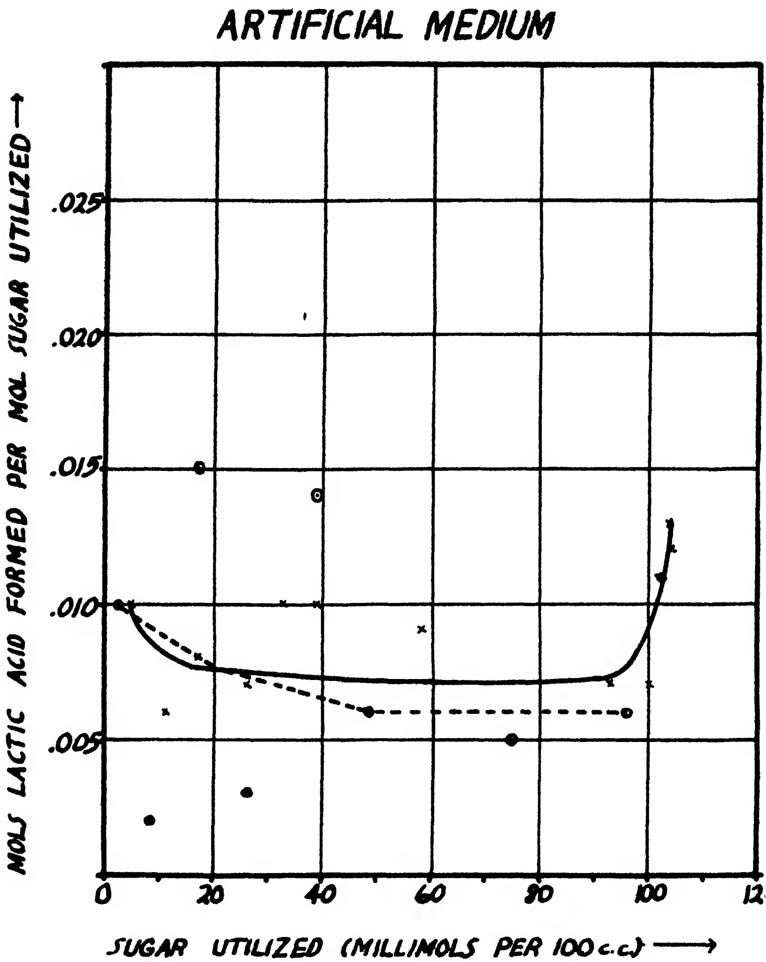

FIG. 4. Relation between lactic acid formed and sugar utilized by Frei Brothers' yeast in artificial medium.

X = ratio of total mols lactic acid formed per total mols sugar utilized.

O = differential of increase in mols lactic acid formed to decrease in mols sugar utilized.

EFFECT OF CARBON SOURCE AND NITROGEN SOURCE UPON LACTIC ACID FORMATION

To determine the effect of carbon source and nitrogen source upon lactic acid formation, several natural and artificial media were fermented with three of the yeasts (66, Champagne, and 2368). The natural media were white and red grape juice, diluted white grape concentrate, and unhopped beer wort. For the artificial media, the basic medium was modified. In

TABLE V

EFFECT OF CARBON AND NITROGEN SOURCE UPON LACTIC ACID PRODUCTION

Carbon source	Nitrogen source	Sugar utilized*	Lactic acid formed†	Total fixed acid formed†	Ratio: Equiv. lactic acid formed / Mols sugar utilized
		Yeast 66			
		m. mols	m. eq.	m. eq.	
White grape juice	White grape juice	120	0.26	1.90	0.016
Red grape juice	Red grape juice	112	−0.71	0.95	0.008
Diluted grape concentrate	Diluted white grape concentrate	110	0.92	0.98	0.009
Wort	Wort	81	0.57	0.60	0.007
Cerelose	Liebig's beef extract	109	2.23	2.15	0.019
Cerelose	Peptone	110	1.49	1.25	0.011
Cerelose	Urea	110	1.41	1.39	0.012
Cerelose	Asparagine	111	1.16	1.45	0.013
Cerelose	l-Aspartic acid	110	−0.32	1.28	0.011
Cerelose	β-Alanine	14	1.50	0.23	0.016
Cerelose	dl-Alanine	110	2.01	1.39	0.013
Cerelose	dl-Leucine	110	1.73	1.53	0.014
Cerelose	Glycine	81	1.75	0.74	0.009
Cerelose	$(NH_4)_2SO_4$	95	1.56	0.90	0.009
Dextrose	$(NH_4)_2SO_4$	86	1.36	0.66	0.007
Levulose	$(NH_4)_2SO_4$	93	1.76	1.42	0.015
d-Galactose	$(NH_4)_2SO_4$	63	2.32	0.75	0.012
Maltose	$(NH_4)_2SO_4$	75	2.24	0.82	0.011
Sucrose	$(NH_4)_2SO_4$	97	1.82	0.90	0.009
		Champagne yeast			
White grape juice	White grape juice	120	0.58	1.85	0.015
Red grape juice	Red grape juice	112	−1.42	1.87	0.016
Diluted grape concentrate	Diluted white grape concentrate	110	0.38	1.31	0.012
Wort	Wort	81	0.37	0.42	0.005
Cerelose	Liebig's beef extract	91	1.90	1.95	0.021
Cerelose	Peptone	110	0.61	1.04	0.009
Cerelose	Urea	110	1.45	1.01	0.009
Cerelose	Asparagine	110	1.24	1.17	0.010
Cerelose	l-Aspartic acid	109	−0.17	1.50	0.014
Cerelose	β-Alanine	8	1.00	0.26	0.032
Cerelose	dl-Alanine	110	1.67	1.14	0.010
Cerelose	dl-Leucine	106	1.49	0.84	0.007
Cerelose	Glycine	50	3.74	0.27	0.005
Cerelose	$(NH_4)_2SO_4$	65	1.15	0.44	0.007
Dextrose	$(NH_4)_2SO_4$	80	1.42	0.60	0.007
Levulose	$(NH_4)_2SO_4$	89	1.59	0.71	0.008
d-Galactose	$(NH_4)_2SO_4$	3			
Maltose	$(NH_4)_2SO_4$	59	1.62	0.82	0.014
Sucrose	$(NH_4)_2SO_4$	65	1.26	0.53	0.008

TABLE V—(*Continued*)

Carbon source	Nitrogen source	Yeast 2368			
		Sugar utilized*	Total fixed acid formed†	Lactic acid formed†	Ratio: Equiv. lactic acid formed / Mols sugar utilized
		m. mols	*m. eq.*	*m. eq.*	
White grape juice	White grape juice	113	0.62	1.32	0.012
Red grape juice	Red grape juice	111	−1.29	1.13	0.010
Diluted grape concentrate	Diluted white grape concentrate	110	0.90	1.24	0.011
Wort	Wort	87	0.87	0.80	0.009
Cerelose	Liebig's beef extract	103	2.34	1.63	0.015
Cerelose	Peptone	107	0.28	0.77	0.007
Cerelose	Urea	80	0.81	0.36	0.004
Cerelose	Asparagine	82	0.71	0.75	0.009
Cerelose	l-Aspartic acid	80	−0.14	0.62	0.007
Cerelose	β-Alanine	11	1.28	0.28	0.025
Cerelose	dl-Alanine	75	1.64	0.66	0.008
Cerelose	dl-Leucine	84	1.50	0.65	0.007
Cerelose	Glycine	71	1.66	0.59	0.008
Cerelose	$(NH_4)_2SO_4$	50	1.00	0.60	0.012
Dextrose	$(NH_4)_2SO_4$	50	0.18	0.56	0.011
Levulose	$(NH_4)_2SO_4$	52	1.49	0.61	0.011
d-Galactose	$(NH_4)_2SO_4$	27	1.40	1.00	0.037
Maltose	$(NH_4)_2SO_4$	38	1.10	0.71	0.018
Sucrose	$(NH_4)_2SO_4$	48	0.17	0.50	0.010

* Milli-mols per 100 ml.
† Milli-equivalents per 100 ml.

one series the carbon source was always 200 gm. of cerelose (about 112 milli-mols dextrose) and the nitrogen source was varied so that the total amount of nitrogen added was always the same. In another series the nitrogen source was always three grams of ammonium sulphate per liter and the carbon source was varied, always using 200 gm. per liter, of the respective sugar. The sugar was determined in the initial and final media and was expressed, in all instances, as dextrose. Expressed in this manner, the initial sugar contents of this series of media were: dextrose 98, levulose 104, d-galactose 83, maltose 80, sucrose (inverted by acid hydrolysis) 117 milli-mols per 100 ml.

In table V the sugar utilized, total fixed acid, and lactic acid formed in the respective media are presented. A study of these data indicates that the amount of lactic acid formed by a given strain of yeast varies with the carbon source and with the nitrogen source. For a given medium there was also considerable variation in the amounts of lactic acid formed by the different organisms. The largest amounts of lactic acid per mol of sugar utilized were formed by all the organisms in the Liebig's beef medium, in

β-alanine, and by yeast 2368 in d-galactose. In the grape media the quantities of lactic acid formed were also fairly high.

In some of the fermentation, the "total fixed acid formed" was less than the lactic acid formed. This was probably due to utilization of some of the fixed acids.

Effect of oxygen supply upon lactic acid formation

Two series of the basic artificial media were fermented by the seven strains of yeast, using mercury fermentation bungs on one lot, and cotton plugs on another. These were fermented for two months with the results shown in table VI. The initial dextrose content of this medium was 110

TABLE VI

Effect of oxygen supply upon lactic acid formation

Oxygen supply	Yeast	Sugar utilized*	Lactic acid formed†	Ratio: $\frac{\text{Equivalent lactic acid}}{\text{Mols sugar utilized}}$
		m. mols	*m. eq.*	
Cotton plug	Burgundy	87	1.06	0.012
Hg bung	Burgundy	85	0.95	0.011
Cotton plug	Frei Brothers'	89	0.91	0.012
Hg bung	Frei Brothers'	90	0.90	0.010
Cotton plug	Tokay	91	1.12	0.012
Hg bung	Tokay	89	0.85	0.009
Cotton plug	2338	93	0.71	0.007
Hg bung	2338	94	0.76	0.008
Cotton plug	66	87	0.82	0.009
Hg bung	66	87	0.81	0.009
Cotton plug	Champagne	65	0.44	0.007
Hg bung	Champagne	78	0.80	0.010
Cotton plug	2368	50	0.56	0.011
Hg bung	2368	60	0.60	0.010

* Milli-mols per 100 ml.
† Milli equivalents per 100 ml.

milli-mols per 100 ml. It may be noted that sugar utilization was incomplete in all of these fermentations. In general, the quantities of lactic acid formed by these seven yeasts varied so little under these two types of conditions, particularly in relation to the sugar utilized, that it may be concluded that formation of this acid is unaffected by the oxygen supply within these limits. Comparison of these data with those given in table IV for the final samples, indicates that a more liberal supply of oxygen might favor lactic acid production. The periodic withdrawal of samples from the fermentations shown in table IV brought about greater aeration than was possible in a fermentation which was simply cotton-stoppered and left undisturbed throughout its entire course.

The quantities of lactic acid found in this investigation are in close agreement with those reported by DURMISHIDZE (5) but they are about two to four times as great as those reported by ESAU (7) for yeast 66, during a ten-day fermentation period. ESAU's media contained 0.5 gm. of peptone and only 10 gm. dextrose per 100 ml. The amounts of lactic acid formed by yeast 66 in ESAU's experiments did not differ greatly in media buffered at pH 4.0, 6.0, and 8.0.

Of the seven yeasts studied here, Champagne yeast was the one which generally produced the least lactic acid, both in absolute amount and with respect to the sugar utilized.

Summary

1. The formation of lactic acid in the fermentation of natural and artificial media was investigated under various conditions.

2. During the course of fermentation of grape and artificial media, lactic acid accumulated at a relatively constant rate throughout the period of sugar utilization, indicating a close relationship between lactic acid formation and sugar utilization. The behavior of all seven organisms was very similar in these fermentations.

3. With a variety of carbon sources and nitrogen sources, the quantities of lactic acid formed were found to depend upon the composition of the media and upon the strain of yeast used.

4. No appreciable difference in lactic acid production was observed between fermentations with cotton stoppers and those with mercury bungs.

5. In general, these results confirm the earlier observations that lactic acid is an important by-product of alcoholic fermentation by pure yeast.

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PRESSURE–COMPOSITION RELATIONSHIPS OF THE GAS IN THE MARINE BROWN ALGA, *NEREOCYSTIS LUETKEANA*

GEORGE B. RIGG AND LYLE A. SWAIN

(WITH FIVE FIGURES)

Introduction

Analysis of the gas contained in the cavities of plants has attracted the attention of many investigators. Such analyses have usually been in relation to photosynthetic and respiratory exchanges, and therefore have considered only oxygen and carbon dioxide, residual gas being termed "nitrogen." The presence of 1 to 12 per cent. of carbon monoxide was reported in the marine brown alga *Nereocystis luetkeana* by LANGDON (**5**) and confirmed by LANGDON and GAILEY (**6**). This seems to be the first and only recorded instance of carbon monoxide being produced by a plant, which therefore merits further study, to which this paper is a contribution.

It is of interest to note that the presence of carbon monoxide was claimed in the blood of mammals, including man, in 1898 by SAINT-MARTIN (**20**) and by DESGREZ and NICLOUX (**1**), and in a series of papers by NICLOUX (**11, 12, 13, 14, 15**) who considered the carbon monoxide to be a normal product of metabolism. Several investigators have reported its presence in small quantities in human blood, including McINTOSH (**10**), RATHERY *et al.* (**16, 17**), GETTLER and MATTICE (**4**), LOEPER *et al.* (**7, 8, 9**), ROFFO (**19**) and SCHMIDT (**21**).

Nereocystis luetkeana has been described by LANGDON but its main characteristics may be recapitulated. It is an annual found only in the sublittoral zone along the west coast of North America. The plant consists of a branched holdfast adhering to the rocks, a long, slender stipe extending from the holdfast to the water surface, and long, ribbon-like fronds attached to the free end of the stipe. The cylindrical stipe is the portion of particular interest. Its growth is very rapid (SHELDON) which results in the attainment of an extreme length of 20 to 25 meters in a single season. Its circumference gradually increases with distance from the holdfast, the stipe terminating in a bulb-shaped end 5 to 10 cm. in diameter. With this increase in circumference a longitudinal cavity develops in the center, leaving a surrounding wall 0.5 to 1.5 cm. in thickness. This hollow portion, or pneumatocyst, is buoyed up by the gas within it. The pressure and composition of this gas are the subject of the present study.

FRYE (**3**) found in the examination of a thousand specimens that the average pressure of the gas in the pneumatocyst was 77 mm. of mercury less than atmospheric pressure, the pressure ranging from 316 mm. less to 124 mm. greater. He showed that the pressure in a specimen was highest at 3–4 P.M. and lowest at 7 A.M., averaging 16 mm. difference.

Analysis of the gas in the cavity was first undertaken by ZELLER and NEIKIRK (**23**), who reported the presence of oxygen, carbon dioxide and nitrogen. Their work was not confirmed by LANGDON who analyzed the gas from a thousand specimens and found that it contained 1 to 12 per cent. carbon monoxide with an average of 4 per cent., and 15 to 24 per cent. oxygen with an average of 18 per cent., and occasional traces of carbon dioxide. He found no indication of hydrogen, methane, or ethylene. The presence of carbon monoxide was demonstrated by spectroscopic examination of blood exposed to the gas and by examination of the external and internal appearance of a guinea pig, a chicken, and a canary, all killed by inhaling the gas. In an experiment to investigate possible changes in the gas composition with the time of day, he collected 4 to 6 specimens at the end of each 4-hour interval, approximately, for a 24-hour period and analyzed their gaseous contents. The specimens of each group, however, showed so much difference among themselves that their average values were of no use in discovering any trend.

LANGDON and GAILEY concluded that the carbon monoxide was present as a result of the respiration of the plant. They found that it was produced in the absence of light; that if the gas in the cavity was replaced with air, carbon monoxide was redeveloped within a few days; and that if the cavity was filled with pure nitrogen or hydrogen, no carbon monoxide was produced. Further, a killed plant produced no carbon monoxide and autolysis of plant tissue produced only carbon dioxide and hydrogen.

RIGG and HENRY (**18**) found the inner surface of the pneumatocyst wall to be sterile, dispelling the possibility that the carbon monoxide might be of bacterial or fungal origin.

The work on Nereocystis reviewed above was done at the Oceanographic Laboratories (formerly the Biological Station) of the University of Washington at Friday Harbor, San Juan Island. The present investigation was carried out in the summers of 1937 and 1938 at the same location. It was planned to repeat some of the earlier work on Nereocystis, with the thought in mind that the changes in gas pressure shown by FRYE might be a reflection of changes in gas composition. As mentioned above, analyses of the gas from a small group of specimens collected at successive time intervals failed to test this hypothesis. The following experiments attempt to follow changes in gas composition by the analyses of a series of small samples taken from a given specimen at successive intervals of time.

Experimental

The apparatus used to follow the pressure variations was a modification of that described by FRYE. The essential part, of all-glass construction, shown in figure 1, was mounted on a board. The board in turn was mounted

on a sturdy raft 8 ft. by 10 ft. so that the apparatus could be moved vertically on slides, and clamped where desired, rigid with respect to the raft. Directly below the tip A (fig. 1), a specimen could be fastened firmly to the raft with the pneumatocyst in a horizontal position at the water level, where wave action kept it continually wet. Before a specimen was clamped in position it was loosened from the bottom to prevent entanglement of the stipe and anchor chain. Frequently the stipe broke when a plant was pulled up, but only those were used in which the break was close to the holdfast far below the beginning of the cavity so that there was no leakage in or out. As was shown by Miss Fallis (2), Nereocystis is not affected by loosening the holdfast or breaking the stipe as long as the plant remains immersed in sea water. It was felt that this arrangement, when the raft was anchored in the harbor, retained the natural habitat of the plant.

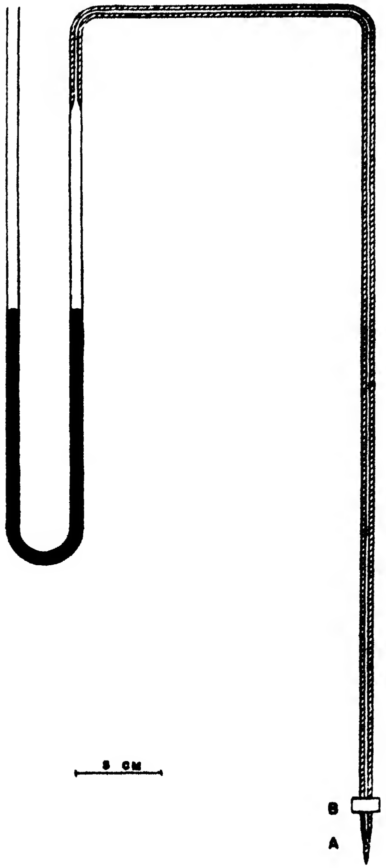

Fig. 1. Mercury manometer.

With the specimen in place, the manometer was lowered, causing the perforated tip A to penetrate the wall of the cavity and the vaselined collar B of rubber pressure tubing, about 2 cm. from the tip, to form a seal which remained airtight for several days. The difference between atmospheric pressure and the pressure in the pneumatocyst was thereby immediately and continuously registered and was observed at frequent intervals. The temperature of the bathing sea water was noted at the same time. The atmospheric pressure at the time of observation was taken from a recording aneroid barometer, checked weekly with a calibrated cistern barometer. The absolute internal pressure in mm. of mercury was thus readily determined.

Successive samples of gas were satisfactorily withdrawn from a given specimen by the use of a 25-ml. hypodermic syringe lubricated with Lubriseal. The needle, filled with distilled water to prevent air-contamination of the sample, was inserted into the plant cavity through a thick layer of vaseline stiffened with 10 per cent. beeswax. A water drop was expelled within the cavity to clear the clogged needle tip, and then a 15-ml. gas sample was withdrawn. On removal of the instrument from the tissue, care was taken to seal the needle tip and the wound through the cavity wall by a slight lateral motion of the needle in the vaseline-beeswax mixture, temporarily softened by means of a hot glass rod. As a further precaution against contamination of the sample from the air, immediately after sealing the needle tip the pressure on the sample was increased to slightly greater than atmospheric. The gas sample was then transferred to an analytical apparatus, designed by GOODMAN and SWAIN (unpublished), by release under mercury.

Vaseline does not stick to the surface of the plant because of its slimy secretion of algin. It was found that heating the surface with an alcohol lamp caused it to remain dry and enabled the vaseline to adhere readily. Examination of the heated tissue after several days showed that the green discoloration (due to destruction of the brown pigment fucoxanthin, leaving the chlorophyll apparent) extended inward about 2 to 3 mm.; the remaining centimeter of tissue was brown and firm and appeared unharmed.

Similar observations on gas pressure and composition were made in the laboratory on specimens placed in a large tank of circulating sea water under controlled light intensities.

Results

The internal pressure was found to be less than atmospheric in the 52 specimens examined in the two summers, varying from 511 mm. to 713 mm. of mercury absolute pressure with an average of 604 mm., all uncorrected for temperature, which ranged from 10° to 14° C. The pressures found by FRYE lie within the limits of 444 mm. and 884 mm. absolute pressure with an average of 699 mm., on the assumption of an atmospheric pressure con-

stant at 760 mm. His values exhibit a similar but wider range than do those reported here, as might be expected from the smaller sampling used in the present study. They differ in that pressures exceeding atmospheric were recorded. Again confirming FRYE's observations, the pressure in any one specimen was found to be lowest in the morning, increasing during the day to a maximum value in the afternoon, and decreasing during the night. Figure 2 shows several typical curves obtained by plotting the absolute internal pressure, calculated to constant temperature, against time. FRYE suggested that this pressure variation might be caused by temperature fluctuations of the sea water. While it is true that the water is somewhat warmer during the day than at night, this merely accentuates slightly the pressure change, which figure 2 clearly shows, is a characteristic of the plant itself.

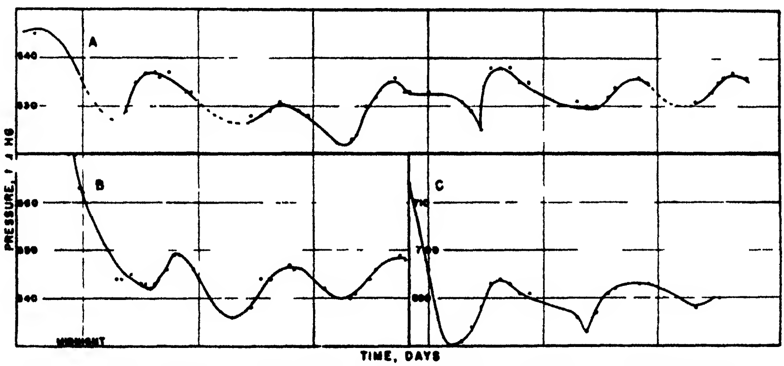

FIG. 2. Daily changes in internal pressure in Nereocystis. Vertical lines represent successive midnights.

The nightly decrease in gas pressure suggested an experiment to determine the effect of continuous darkness. In specimens placed in the laboratory tank of sea water in a dark room for several days, the pressure decreased throughout the time of the experiment. Typical results are shown in A and C of figure 3.

Since the pressure in the plant is different from that of the atmosphere, it follows that the pneumatocyst tissue is comparatively impervious to air. This was demonstrated in the laboratory by evacuating the bulb-shaped end cut from a pneumatocyst. A vacuum could be held as long as 24 hours before softening of the tissue at the cut surface caused leakage. When carbon dioxide was introduced into a fresh, evacuated bulb the pressure decreased rapidly as shown in B of figure 3, indicating the removal of the gas from the interior of the plant. LANGDON and GAILEY noted a similar reduction in pressure when hydrogen was introduced.

Analysis of samples of gas taken from a specimen immediately after

inserting the manometer showed a range of 17.8 to 24.6 per cent. oxygen, 0.8 to 7.6 per cent. carbon monoxide and 0 to 0.3 per cent. carbon dioxide (4 of 7 specimens so examined contained no carbon dioxide). These results confirm LANGDON's data except for the increased frequency of small amounts of carbon dioxide. In table I are given the results of the analysis of samples of gas removed over a period of time from three specimens exposed to different light conditions. It is readily seen that in specimen A, kept on the raft,

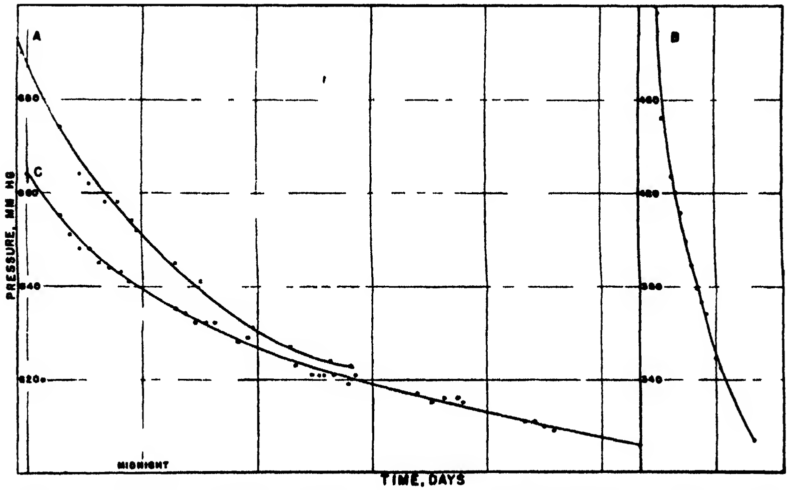

FIG. 3. A and C. Pressure change in Nereocystis in darkness. B. Pressure change in Nereocystis bulb containing only carbon dioxide.

the oxygen concentration increased during the day and decreased during the night, while the carbon dioxide behaved in the reverse direction. In specimen C, kept in the salt water tank in the dark room, the concentration of oxygen steadily decreased and that of carbon dioxide increased. Under a constant illumination of 300 foot-candles, obtained by two 200-watt electric lights in the dark room, specimen B behaved as though it were in total darkness.

These results are also presented in figure 4, accompanied by the pressure curve of each specimen. The vertical lines in the pressure curve represent the decrease in pressure resulting from the removal of a sample of gas. In one case in specimen A it represents an increase in pressure due to leakage of air into the plant when the sample was taken. Each gas curve indicates the change in quantity of that gas in the plant from immediately after a sample was removed until the next was taken, expressed in milliliters. For example, when the first sample was taken from A, the gas contained 20.62 per cent. oxygen, the pressure after removal of the sample was 634 mm. mercury, and the temperature was 11.6° C., from which it is found that the

specimen at that time contained 151.1 ml. of oxygen at standard conditions. When the second sample of gas was taken, the pressure was 639 mm., the temperature was 11.2° C., and the gas contained 21.22 per cent. oxygen, which represents 157.0 ml. oxygen at standard conditions. The oxygen curve shows this increase in content of 5.9 ml. In both specimens A and C the volume of the third sample removed was estimated, and the volume of entering air was calculated, to determine the changes in quantity of the several gases. In C the volume of entering air equalled the volume of the sample removed.

TABLE I

CHANGES IN GAS COMPOSITION IN NEREOCYSTIS AT SEVERAL LIGHT INTENSITIES

SPECIMEN	DATE IN AUG. 1938	TIME	CO_2	O_2	CO
			%	%	%
A Daylight. Internal volume = 930 ml.	8	9:30	0.59*	20.62	1.35
		16	0.35	21.22	1.45
	9	7	0.54	19.76	1.00
		17	0.04	20.83	1.33
	10	8	0.72	19.78	1.39
		16:30	0.21	20.58	1.30
B 300 ft.-candles. Internal volume = 1080 ml.	6	19	0.00	22.70	0.00
	7	8	0.58	20.34	0.34
		17:30	1.11	19.00	0.11
	8	8	1.02	17.49	0.33
		19	1.23	16.29	0.39
	9	10:30	1.27	15.06	0.36
C Darkness. Internal volume = 610 ml.	1	16:30	0.00	17.84	1.74
		23	0.68	16.76	1.42
	2	7	1.16	14.69	1.89
		16:30	1.44	12.73	1.78
	3	9	1.71	9.69	2.02

* Sample removed 14 hours after the pressure manometer was inserted in the specimen.

The pressure curve of A shows an increase during the day and a decrease during the night, agreeing with the results shown in figure 2. The quantity of carbon dioxide increases during the night and decreases during the day, but not in sufficient amount to compensate for the opposite change in oxygen content. In B and C, in weak light and darkness, respectively, the pressure decreases continuously as does the oxygen content. The carbon dioxide content increases, but at a much slower rate than the oxygen decreases.

In all specimens, the quantity of carbon monoxide remained the same, within experimental error.

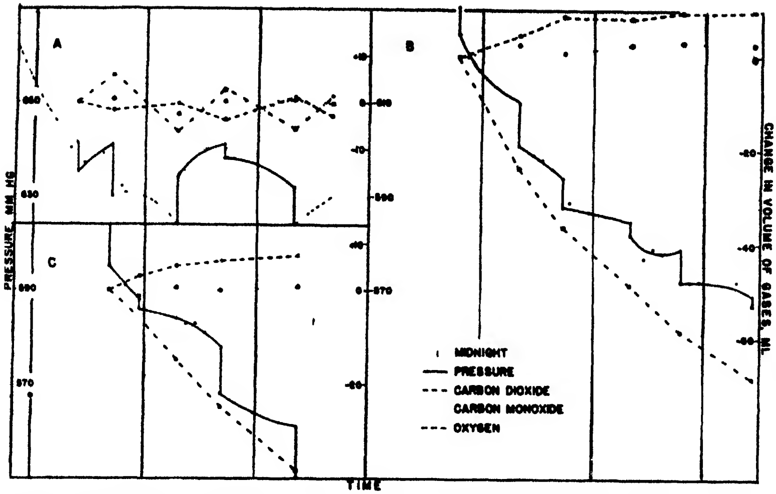

FIG. 4. Changes in pressure and quantities of gases in Nereocystis. A. On raft; B. 300 ft.-candles; C. Darkness.

Discussion

The foregoing results suggest the following hypothesis: The changes in internal pressure are a reflection of changes in the composition of the gas within the plant. Of the gases present, carbon dioxide occurs infrequently and then in very small amounts, the carbon monoxide content seems to remain constant (as shown in fig. 4), and nitrogen is not affected by respiration or photosynthesis. The changes in pressure therefore reflect changes in the amount of oxygen. The removal of oxygen from the specimens in darkness or weak light by respiration, possibly accentuated by injury, parallels the observed decrease in pressure. This decrease in oxygen content in Nereocystis in darkness corroborates LANGDON and GAILEY who showed that the oxygen content of uninjured plants kept several days in light-proof boxes in the harbor was far below the average value. Under normal conditions the decrease in oxygen content at night, caused by respiration, is accompanied by a decrease in pressure, and the increase in the amount of oxygen during daylight hours, caused by photosynthesis, is accompanied by an increase in pressure. Apparently the oxygen is used up in respiration and no corresponding volume of free gas is produced in the pneumatocyst. This might be due to the use of some compound of low oxygen content as respiratory material or to the absorption of the produced gas by the tissue of the pneumatocyst wall.

The development of carbon dioxide in all specimens under extended observation is possibly caused by injury to the plant by the clamps, the tip of the manometer, the needle of the syringe, or the heating necessary to

make the vaseline adhere. LANGDON and GAILEY showed, however, that it developed in their light-proof boxes. It is possible that excessive amounts of carbon dioxide cannot be readily removed from the gas space by the plant. In any event its frequent absence in the normal plant makes inseparable the changes in gas pressure and the oxygen content.

The rapid growth of Nereocystis, considered in relation to the impermeability of the tissue to air, suggests that the observed pressure is below atmospheric because air cannot enter the pneumatocyst cavity sufficiently rapidly to maintain atmospheric pressure. An observation giving support to this idea was made on *Macrocystis pyrifera,* growing in Neah Bay, Washington. This is a large brown alga closely related to Nereocystis. It consists of a holdfast and several long, cord-like stipes, to which are attached large broadly lanceolate fronds. At the base of each frond is a hollow cyst, corresponding to the pneumatocyst of Nereocystis. Growth occurs at the free end of the stipe, where new fronds and cysts develop.

Pressure in each cavity on several stipes was determined using an apparatus similar to that shown in figure 1, but constructed on a much smaller scale of fine bore capillary glass tubing. The results are shown in figure 5,

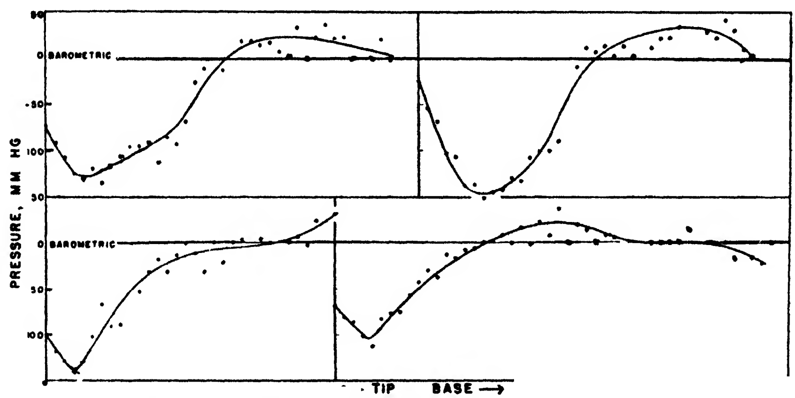

FIG. 5. Pressure in successive cysts of Macrocystis.

in which the pressure in each cavity, commencing at the free end, is designated by a point. Omission of a point indicates a broken cyst. It is evident that the region of rapid growth is characterized by pressures lower than atmospheric, whereas the pressures in the older, more mature region exceed atmospheric. All the specimens of Nereocystis observed in this study were vigorously growing plants.

Summary

1. The composition and pressure of the gas in the pneumatocyst of the marine brown alga, *Nereocystis luetkeana,* have been studied on single specimens for several days.

2. The daily change in pressure, from a maximum in the afternoon to a minimum in the morning, is confirmed.

3. The presence of oxygen and carbon monoxide and frequent absence of carbon dioxide (in normal specimens) are confirmed.

4. In plants in the sea the oxygen content increased during the day and decreased at night, corresponding with the pressure change.

5. In plants maintained in darkness, there was a continuous decrease in pressure and in oxygen content and a development of carbon dioxide.

6. The hypothesis is advanced that the change in pressure of the gas is caused by the change in its oxygen content.

7. From a brief study of the pressure in the cysts of *Macrocystis pyrifera,* the suggestion is made that the reduced pressures observed in both it and Nereocystis are the result of their rapid growth.

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X-RAY EFFECTS ON THE GROWTH AND REPRODUCTION OF WHEAT

D. J. WORT

(WITH FOUR FIGURES)

Introduction

Evidences of modification and injury to plant parts are numerous in the extensive literature on the effects of x-ray treatment of plants. JOHNSON (2) lists the effects produced on seventy species of flowering plants. Her work with wheat (3) shows that growing material is more strongly affected than non-growing by equal doses of radiation given in the same way. Seedlings from soaked grains given treatments of 1000 r-units and 5000 r-units made less growth in all respects than did controls, but showed increased tillering. No flowering data were included.

SHULL and MITCHELL (4) obtained deleterious effects when unfiltered radiations were used but the use of metallic screens, high voltage, low amperage, and brief exposures giving approximately 100 r-units, resulted in stimulative action on wheat, corn, oats, and sunflower seedlings. Twenty-four-hour-old wheat seedlings were irradiated with doses of x-rays varying from 565 to 13,560 r-units by FRANCIS (1). Retardation of fresh weight and dry weight production by the growing parts of the seedlings and retardation of linear growth of the coleoptile, the leaf, the primary root, and the lateral roots was obtained with all doses and time intervals employed. Respiration was likewise depressed with the exception of those seedlings which had received the smallest dose of radiation (565 r-units) and which were tested 5 or 6 hours after irradiation.

The procedure of SHULL and MITCHELL was closely followed in an attempt to obtain further data on the stimulative effects of filtered x-radiation on growth, height, tillering, heading and flowering, green and dry weights, and moisture content of Marquis spring wheat. An attempt to determine the influence of age of seed on response to irradiation was made by the use of two samples of seed; one 57 months old (4 years, 9 months), the other 9 months old. A preliminary trial was made with Fulhio winter wheat.

Methods

Two different samples of Marquis spring wheat seed were used, one 57 months old, the other 9 months old. One lot of older seeds was irradiated on March 14 (experiment 1) and a second lot on March 26 (experiment 2). The lot of seed 9 months old was x-rayed on April 3, 1940. Although treated on the same day the two groups (experiments 3 and 4) were irradiated separately. Fulhio winter wheat was x-rayed and planted on April 16, 1940.

An amount of water equal to 60 per cent. of the air-dry weight of the seed was added to the wheat in Petri dishes and the seed was allowed to germinate for 24 hours at 21° C. At the end of this period the radicles were about 3 mm. long. In selecting grains for irradiation, care was taken to choose those with radicles of uniform length.

Thirty-five seedlings were placed in a Petri dish for irradiation. Seven such groups were x-rayed at a distance of 30 cm. from the target, using a 1-mm. aluminum screen. The x-ray tube was a standard, air-cooled Coolidge Victor tube and was maintained at a potential of 100 pk. kilovolts and a current of 5 milliamperes. At this setting the output of the machine was approximately 38 roentgens per minute. The seven exposures were for 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 6.0 minutes which gave doses of 19, 38, 57, 76, 114, 152 and 228 r-units respectively. One group was not irradiated and served as control.

Seven seeds of the irradiated or control grain were planted in each of five 4½-inch pots filled with well mixed garden loam. All plants were given a 16-hour photoperiod, natural daylight being supplemented by 200-watt mazda lamps suspended 24 inches above the tops of the pots. The pots were placed in different positions once a week to obtain identical illumination as far as possible. Measurement of height, from the ground level to the tip of the longest leaf on the leading tiller, was made 14, 21, and 28 days after planting. Six weeks after planting 18 plants were cut at the ground level and green weights, dry weights, and moisture content of the tops determined. Observations regarding heading and flowering were made on the remaining 17 plants. The wheat was assumed to have headed when the head was completely out of the sheath and to have flowered when the anthers were first extruded. At the end of the 56 days these remaining plants were cut at the soil level and green and dry weights determined.

The Fulhio winter wheat was measured at the age of 14 and 28 days. All of the winter wheat plants were harvested when 28 days old and green and dry weights determined. Only the data for the control plants and those receiving 114 r-units were recorded.

Results and discussion

Growth, height, and tillering

The effect of irradiation on the growth and height of plants produced by seeds 57 months old is given in table I. It appears that all of the treatments, 19–228 r-units, resulted in a stimulated growth rate and greater height. While doses of 76–228 r-units resulted in increments in growth and height which were nearly equal, the greatest increase in height (12.8 per cent.) attained at the age of 28 days, was produced by a treatment of 114 r-units. The increased growth in all cases was evidenced by greater

TABLE I

HEIGHTS OF X-RADIATED MARQUIS WHEAT PLANTS FROM 57-MONTH-OLD SEEDS. RESULTS ARE AVERAGES FROM 35 PLANTS

EXPERIMENT	AGE	X-RAY DOSE IN R-UNITS							
		0	19	38	57	76	114	152	228
	days	*in.*	*in.*	*in.*	*in.*	*in.*	*in.*	*in.*	*in.*
1	14	6.71	6.80	7.27	7.44	7.35	6.65	6.64	7.02
	21	9.96	9.97	10.36	10.40	10.91	10.36	9.97	9.37
	28	10.62	10.71	11.30	11.30	11.80	11.50	11.87	10.88
2	14	5.14	6.79	5.68	6.44	5.82	7.72	7.39	7.56
	21	8.86	9.65	8.83	9.83	9.71	10.79	10.17	11.38
	28	10.14	10.89	9.74	11.21	11.41	11.93	11.06	12.49
Average of 1 and 2	14	5.92	6.80	6.47	6.94	6.58	7.18	7.01	7.29
	21	9.41	9.81	0.59	10.11	10.31	10.57	10.07	10.37
	28	10.38	10.80	10.52	11.25	11.60	11.71	11.46	11.68

height, larger stem diameter, and larger leaf area. Only height was measured, however. No difference in color was evident and irradiation had no effect on tillering.

Table II presents the response of plants grown from seeds 9 months old.

TABLE II

HEIGHTS OF X-RADIATED MARQUIS WHEAT PLANTS FROM 9-MONTH-OLD SEEDS. RESULTS ARE AVERAGES FROM 35 PLANTS

EXPERIMENT	AGE	X-RAY DOSE IN R-UNITS							
		0	19	38	57	76	114	152	228
	days	*in.*	*in.*	*in.*	*in.*	*in.*	*in.*	*in.*	*in.*
3	14	9.58	10.82	11.64	11.70	11.24	11.30	10.94	10.72
	21	11.86	13.34	13.51	14.12	13.53	13.20	12.77	13.76
	28	12.95	13.36	15.10	15.61	14.84	14.78	14.05	14.09
4	14	10.36	11.06	11.47	11.46	11.23	11.48	11.24	11.44
	21	13.98	14.50	14.65	14.99	15.48	15.51	15.19	14.75
	28	14.22	15.76	15.99	16.47	16.79	17.06	16.43	15.63
Average of 3 and 4	14	9.87	10.94	11.56	11.58	11.24	11.39	11.09	11.08
	21	12.92	13.92	14.08	14.56	14.56	14.36	13.98	14.26
	28	13.59	14.56	15.55	16.04	15.82	15.92	15.24	14.86

As in the former case all treatments resulted in stimulated growth and height. The height curves, shown in figure 1, reveal that the greatest height was obtained in those plants which had received 57, 76, and 114 r-units. The greatest increment in height (18.0 per cent.) was found in plants which had received 57 r-units of x-radiation when 24 hours old. Tillering was stimulated by treatments of 38–114 r-units; the largest average number of

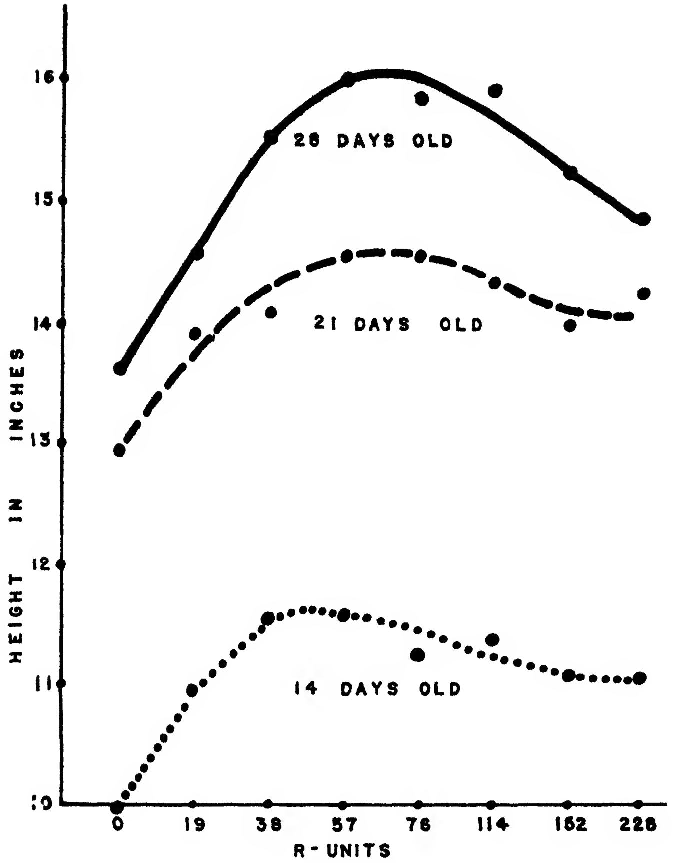

FIG. 1. Heights of Marquis wheat plants when 14, 21, and 28 days old grown from seed 9 months old. The 24-hour-old seedlings were x-radiated with r-unit doses as indicated. Each height is the average for 35 plants (experiments 3 and 4).

culms per plant (1.3) was found on plants irradiated with 76 r-units. Only one head, however, was formed per plant. A decidedly lighter color was observed in all irradiated plants, but the difference was not particularly evident when the plants were 56 days old. As in the experiments with the older seed the stimulus to growth was shown by greater height, stem diameter, and leaf area.

WET AND DRY WEIGHTS; MOISTURE CONTENT

Irradiation of the seedlings from the older seeds resulted generally in increased wet and dry weight; the one exception was an irradiation of 228 r-units which resulted in plants having wet and dry weights equal to those of the controls determined 56 days after planting. Table III gives the

TABLE III

WET AND DRY WEIGHTS AND MOISTURE CONTENT OF X-RADIATED MARQUIS WHEAT PLANTS FROM 57-MONTH-OLD SEEDS. RESULTS FOR PLANTS 42 DAYS OLD ARE THE AVERAGES FROM 18 PLANTS; FOR 56-DAY-OLD PLANTS THE AVERAGES ARE FROM 17 PLANTS

EXPERIMENT	AGE IN DAYS	TEST	X-RAY DOSE IN R UNITS							
			0	19	38	57	76	114	152	228
1	42	Wet weight*	0.44	0.44	0.71	0.69	0.65	0.65	0.51	0.45
		Dry weight*	0.09	0.09	0.13	0.12	0.14	0.11	0.10	0.11
		Moisture†	80.2	80.6	82.0	82.9	78.6	82.6	80.8	75.5
	56	Wet weight	0.50	0.50	0.71	0.77	0.77	0.61	0.61	0.40
		Dry weight	0.15	0.15	0.21	0.21	0.24	0.17	0.19	0.15
2	42	Wet weight	0.54	0.59	0.65	0.57	0.72	0.56	0.64	0.64
		Dry weight	0.10	0.13	0.12	0.13	0.14	0.13	0.13	0.13
		Moisture	82.1	78.7	81.7	77.7	80.4	77.9	79.3	79.7
	56	Wet weight	0.69	0.66	0.81	0.79	0.97	0.69	0.67	0.79
		Dry weight	0.21	0.19	0.20	0.24	0.26	0.22	0.18	0.21
Average of 1 and 2	42	Wet weight	0.49	0.52	0.68	0.63	0.69	0.61	0.58	0.55
		Dry weight	0.09	0.11	0.12	0.12	0.14	0.12	0.12	0.12
		Moisture	81.2	79.7	81.9	80.3	79.5	80.3	80.1	77.6
	56	Wet weight	0.59	0.58	0.76	0.78	0.87	0.65	0.64	0.59
		Dry weight	0.18	0.17	0.21	0.23	0.25	0.20	0.19	0.18

* Given in grams.
† Percentage of wet weight.

weight and moisture data of experiments 1 and 2. The greatest average wet weight per plant and the greatest dry weight were found in those plants which had received 76 r-units. These weights were greater than those of control plants by 40.8 per cent. and 55.5 per cent., respectively. The various irradiations produced little differences in moisture content of the plants. The average weight values for experiments 1 and 2 are shown graphically in figure 2.

The data of table IV indicate that in the case of fresh seed the higher radiation doses resulted in decreased wet and dry weights compared with control plants. Fifty-seven r-units, however, caused an increase of 67.6 per cent. in wet weight and 23.5 per cent. in dry weight. Moisture content

was not significantly affected by irradiation. The average weight values for experiments 3 and 4 are shown graphically in figure 3.

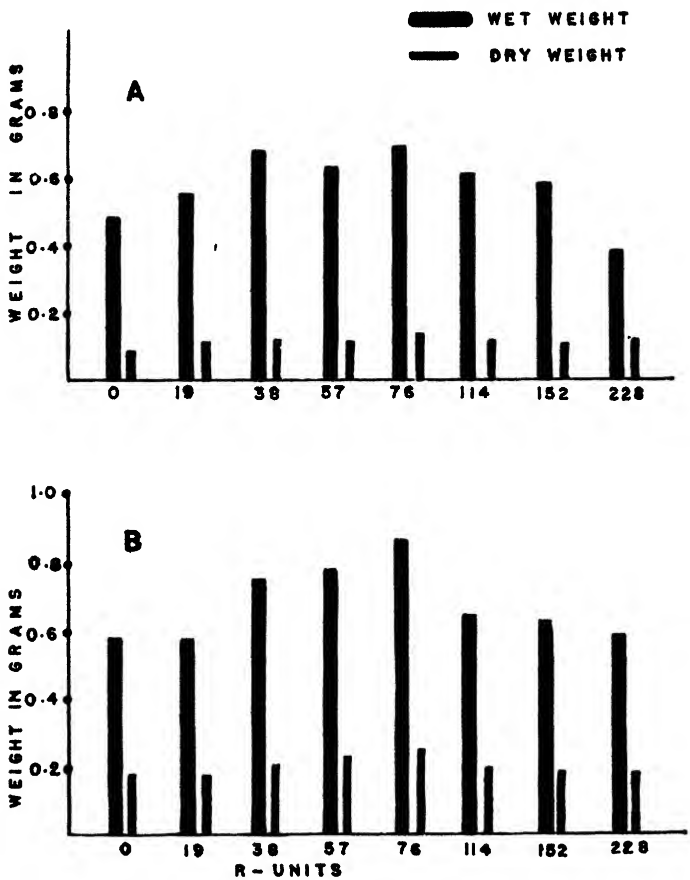

FIG. 2. Wet and dry weights of plants grown from 57-month-old Marquis spring wheat. The 24-hour-old seedlings were given filtered x-ray treatment of the number of r-units shown in the figure. A. Average weights for 18 plants 42 days old. B. Average weights for 17 plants 56 days old.

HEADING AND FLOWERING

An interesting result with respect to heading and flowering is shown by the data of table V. All treatments of the older seed resulted in accelerated heading and flowering, the maximum acceleration being obtained with doses of 76 and 114 r-units. In each case flowering was accelerated by approximately 3 days. The seedlings of the fresh grain, however, were adversely affected by x-radiation, the controls flowering by as much as 6.7 days before the treated plants. That this difference in response may be attributed to

TABLE IV

WET AND DRY WEIGHTS AND MOISTURE CONTENT OF X-RADIATED MARQUIS WHEAT PLANTS FROM 9-MONTH-OLD SEEDS. RESULTS FOR PLANTS 42 DAYS OLD ARE THE AVERAGES FROM 18 PLANTS; FOR PLANTS 56 DAYS OLD, THE AVERAGES ARE FROM 17 PLANTS

Experiment	Age in days	Test	X-ray dose in r-units							
			0	19	38	57	76	114	152	228
3	42	Wet weight*	0.75	0.81	1.28	1.49	1.37	1.14	1.14	0.94
		Dry weight*	0.16	0.16	0.20	0.31	0.21	0.16	0.19	0.16
		Moisture†	77.6	80.4	84.2	79.4	84.5	85.9	83.2	83.3
	56	Wet weight	0.91	0.90	1.70	1.69	1.29	1.20	1.18	1.04
		Dry weight	0.31	0.28	0.42	0.43	0.30	0.25	0.29	0 25
4	42	Wet weight	1.00	1.47	1.33	1.41	1.47	1.57	1.09	0.85
		Dry weight	0.20	0.21	0.19	0.21	0.22	0.23	0.16	0.17
		Moisture	79.9	85.4	86.0	84.9	85.4	85.1	84.9	80.0
	56	Wet weight	1.12	1.25	1.55	1.71	1.47	1.58	1.25	0.33
		Dry weight	0.36	0.31	0.36	0.41	0.34	0.39	0.29	0.27
Average of 3 and 4	42	Wet weight	0.88	1.14	1.31	1.45	1.42	1.36	1.12	0.90
		Dry weight	0.18	0.19	0.20	0.26	0.22	0.20	0.18	0.17
		Moisture	78.8	82.9	85.1	82.2	85 0	85.5	84.1	81.7
	56	Wet weight	1.02	1.03	1.63	1 70	1.38	1.39	1.21	0.94
		Dry weight	0.34	0.30	0.39	0.42	0.32	0.32	0.29	0.26

* Given in grams.
† Percentage of wet weight.

the age of the seed is suggested by the fact that growth conditions for all the experiments were as similar as possible with the exception of the slightly greater intensity and duration of daylight in the case of experiments 3 and 4. These experiments were started April 3 as compared with March 14 and March 26 for experiments 1 and 2 respectively. The photoperiod for all experiments was maintained at 16 hours, however.

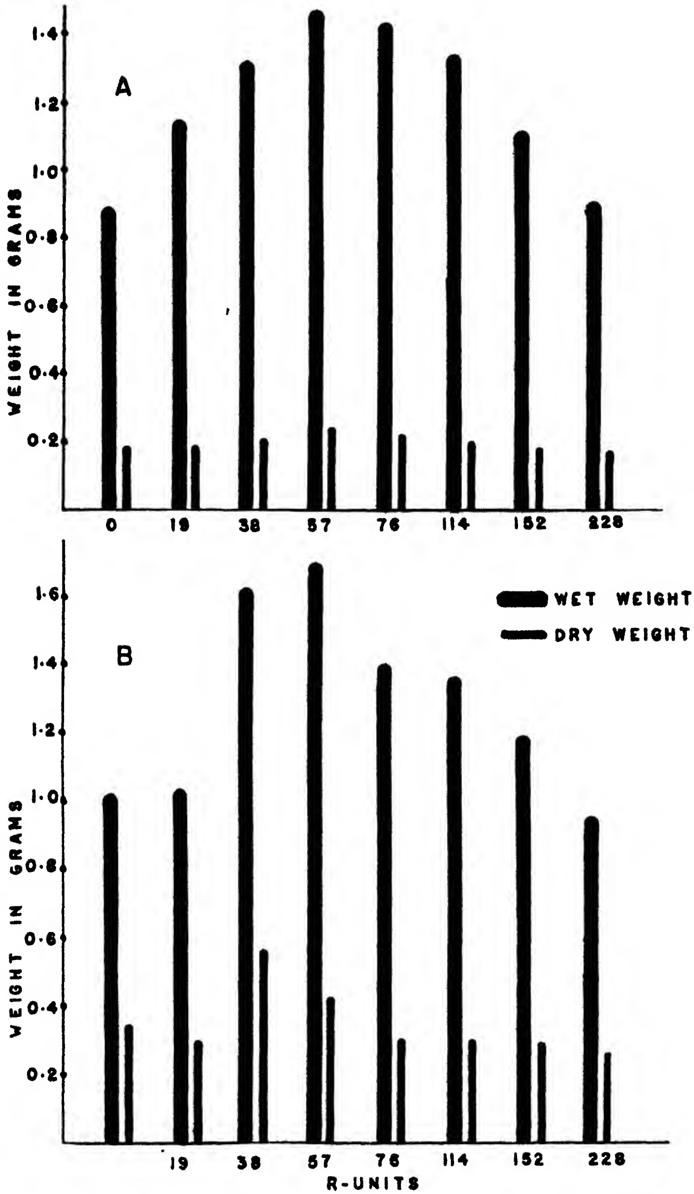

FIG. 3. Wet and dry weights of plants grown from 9-month-old Marquis spring wheat. The 24-hour-old seedlings were given filtered x-ray treatment of the number of r-units shown in the figure. A. Average weights of 18 plants 42 days old. B. Average weights of 17 plants 56 days old.

TABLE V

Number of days from planting to heading and flowering by X-radiated Marquis wheat plants from seed 57 months old (Experiments 1 and 2); and from seed 9 months old (Experiments 3 and 4). Results are the averages from 17 plants

X-ray dosage	Exp. 1		Exp. 2		Average of 1 and 2		Exp. 3		Exp. 4		Average of 3 and 4	
	Head	Flower	Head	Flower	Head	Flower	Head	Flower	Head	Flower	Head	Flower
r-units	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*
0	53.1	53.6	49.8	51.2	51.5	52.4	42.0	43.4	42.0	43.7	42.0	43.6
19	51.1	52.0	49.0	49.1	50.1	50.6	42.7	44.2	49.2	50.5	45.9	47.3
38	49.9	51.1	49.7	50.9	49.8	51.0	46.2	47.0	49.6	50.3	47.9	48.6
57	51.4	52.5	45.4	48.0	48.4	50.3	47.0	48.0	49.2	50.3	48.1	49.1
76	47.9	49.1	48.8	49.7	48.4	49.4	48.0	49.7	47.8	48.2	47.9	48.9
114	49.2	50.8	46.7	48.4	48.0	49.6	48.2	49.8	47.1	48.3	47.6	49.0
152	51.4	53.7	47.2	49.0	49.3	51.4	47.0	48.5	51.2	52.1	49.1	50.3
228	50.5	51.5	47.9	49.2	49.2	50.4	45.3	46.8	45.2	46.7	45.3	46.8

Fulhio winter wheat

Irradiation of 24-hour old Fulhio winter wheat seedlings by doses of from 19 to 228 r-units produced stimulation of growth, compared with that of seedlings receiving no treatment. The greatest effects were obtained with a dose of 114 r-units and only these results are recorded in table VI.

TABLE VI

Height, weight, and tiller number of control and x irradiated Fulhio winter wheat at age 28 days Results are the averages from 35 plants

	Average height/ plant, age 14 days	Average height/ plant, age 28 days	Average wet weight/ plant, age 28 days	Average dry weight/ plant, age 28 days	Average number of tillers/ plant
	in.	in.	gm.	gm.	
Control	8.08	13 76	0.83	0 12	1.00
Irradiated with 114 r-units	10.72	17 47	1 48	0.21	1.14

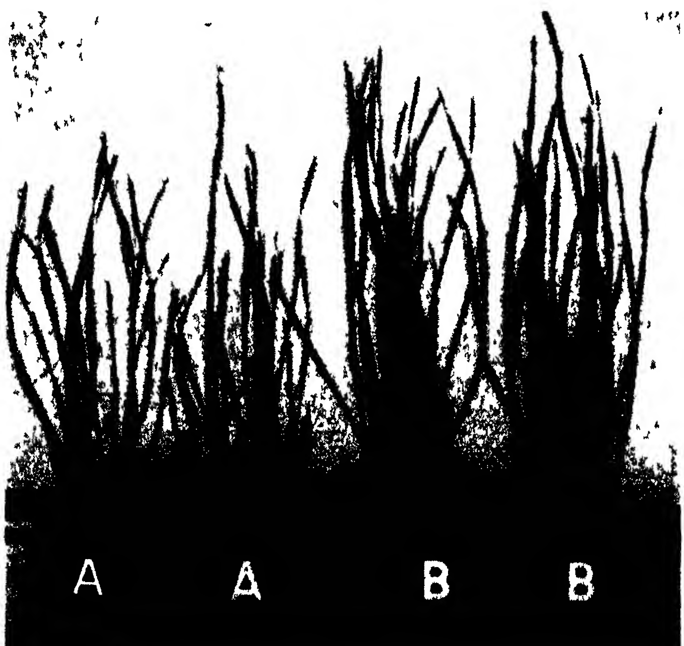

Fig. 4. Irradiated and control Fulhio winter wheat photographed when 28 days old. Those photographed are representative of 35 plants. A. Control. B. Irradiated when 24 hours old with a dose of 114 r-units.

Figure 4 shows the appearance of the wheat when harvested at the age of 28 days.

Summary

1. Twenty-four-hour-old seedlings of Marquis spring wheat grown from seeds 57 months old and 9 months old were irradiated by filtered x-rays of 19 to 228 r-units. Controls were plants receiving no treatment.

2. Irrespective of the age of the seed greatest growth rate and height were produced by 114 r-unit treatments.

3. Greatest fresh and dry weights of plants grown from the older seeds resulted from the use of 76 r-units; of fresh seeds, from 57 r-units.

4. X-radiation of from 76 to 114 r-units accelerated heading and flowering of plants grown from old seed by as much as 3 days, but retardation of heading and flowering occurred when fresh seed was irradiated.

5. Height and weight of Fulhio winter wheat seedlings were increased considerably by all doses of x-rays used, the maxima occurring when the dosage was 114 r-units.

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PLANT CULTURE EQUIPMENT

FRANK M. EATON

(WITH FOUR FIGURES)

Introduction

A detailed description is presented in this paper of small sand and water culture equipment. A number of large out-of-doors sand cultures both for tree and annual crops have also been constructed at the U. S. Regional Salinity Laboratory, Riverside, California. In their general features these latter cultures are like those described in an earlier publication (**3**). The more significant modifications are discussed.

The same principle of operation is used in all of the sand culture equipment. Briefly stated, each sand culture consists of a sand bed and a solution reservoir. Nutrient solution is pumped from the reservoir onto the surface of the sand at hourly or other selected intervals. The solution applied to the surface of the sand displaces to the reservoir a large part of the solution remaining in the sand from the previous flushing. Drainage to the reservoirs is provided by the use of a permeable aluminum silicate material manufactured in the form of bricks for the insulation of furnaces. These bricks (or discs) confine root growth to the sand and thus prevent the stoppage of drains. Inasmuch as the intervals of application of solution to the sand are controlled by time clocks, a minimum of manual attention is required.

Small sand cultures

The stoneware cultures, figures 1 to 3, were designed for nutritional studies in the greenhouses where the effects of a number of treatments are compared in replicated cultures. While the equipment shown was intended primarily for small plants, its usefulness for large plants is indicated by the tomatoes in figure 2. For use with large plants advantages of larger reservoirs are indicated, since the frequency with which new solutions are substituted for old ones, or the frequency with which determinations and additions of nutrient ions are made, can thereby be reduced. The pottery company that has cooperated in the manufacture of the stoneware has advised that they can build 35- or 40-liter stoneware reservoirs with bell-shaped tops that would accommodate the present sand trays and other fittings.

CHAPMAN'S and LIEBIG'S idea (**1**) of using air-lift pumps has been adopted in these cultures. These investigators made use of large earthenware tile reservoirs set in greenhouse floors.

The sand cultures shown in figure 3 are mounted on a rotating table to produce uniform conditions in an unevenly lighted greenhouse. Com-

pressed air is brought into the center of the revolving table through a mercury sealed connection. The revolving portion of the table top rests on the wheel assembly of a heavy truck trailer. The axle of this assembly is set in a concrete-filled excavation beneath the greenhouse floor.

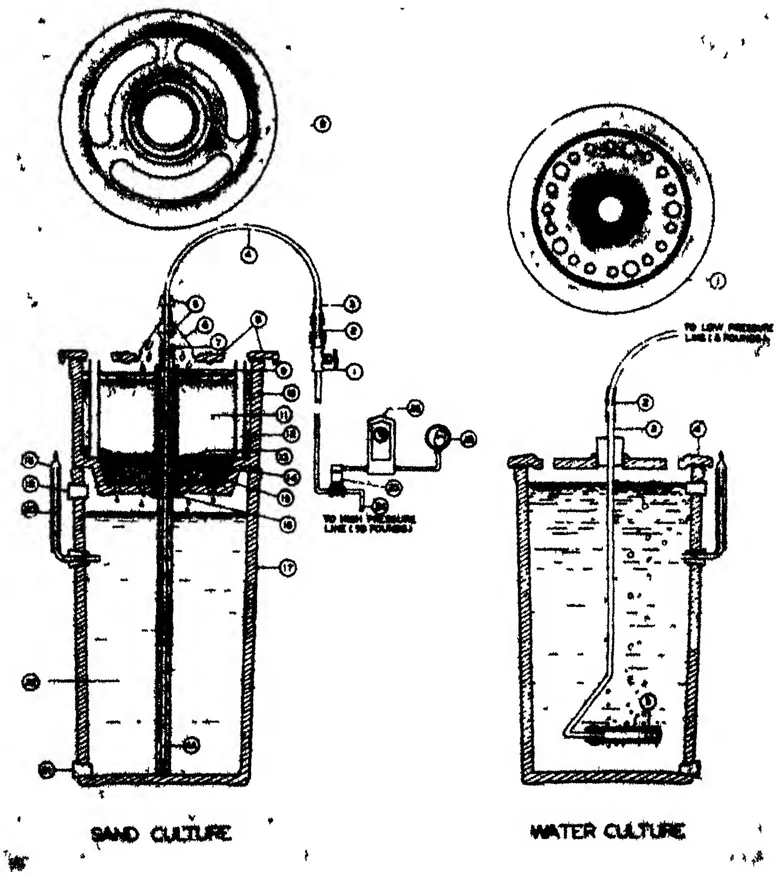

FIG. 1. Cross section view of sand culture (left) and water culture (right).

The following paragraphs, numbered to correspond to the designations of the parts in figure 1, left, describe the character of the various pieces of the sand culture equipment:

1. *Air valve.*—This is a ¼-inch needle valve of the less expensive type. It is used for adjusting the rate of flow of air from the outlet at 4a. The valve has male threads at both ends (fig. 2, left).

2. *Hose connector.*—This ¼-inch acetylene-welder type of connection has

FIG 2 Sand cultures on greenhouse benches

FIG 3 Sand cultures mounted on a rotating table in unevenly lighted greenhouse.

a hexagonal nut which permits ready disconnection of the air line when the cultures are to be dissembled.

3. *Koroseal tubing.*—Tubing of this type[1] (suggested by L. A. RICHARDS) stands up under greenhouse light conditions much better than rubber tubing. It is heated in a water bath before being set in place.

4. *Air-discharge tubes.*—Soft aluminum tubing with ¼-inch outside diameter. A 1/32-inch hole is bored through one wall at 4a to emit air. The lower end is closed by clamping it in a machine vise.

5. *Funnel supports.*—These are the two halves of a no. 2 rubber stopper.

6. *Aluminum funnel.*—Outside diameter of base, 2¾ inches.

7. *Solution delivery tube.*—Hard thin-walled aluminum tubing 22¾ inches long with ¾-inch outside diameter. V-notches ¾-inch deep are cut on opposite sides of the lower end to provide inlets for the solution. The delivery rate of solution by this tubing with an inside diameter of 17 mm. in single tests was 2.6 liters per minute compared with a rate of 1.3 liters per minute from a tube with 13-mm. inside diameter. Corrosion of this tubing can be delayed by dipping it in Bakelite varnish.[2]

8. *Glazed stoneware covers.*—These are used to suppress growth of algae and check surface evaporation. Slips of sheet aluminum or cardboard with U-shaped notches for the plant stems are laid over the openings in the cover after the plants have been thinned to final number.

9. *Glass sleeve and overflow.*—Pyrex glass tubing with inside diameter of 22 mm. and outside 30 mm., length 17 cm. To seal in place, the glass tube is extended through the circular opening in porous disc, 15, and bottom of the tray, 10, letting the lower end of the tube rest on the work bench. After placing a centimeter depth of coarse sand in the bottom of the V-shaped opening around the porous disc, hot G–K compound, 14, is poured in with a spoon until the groove is filled. A thin rim of G–K compound is then built up on the disc around tube. When this material has hardened, the tray is inverted and hot red sealing wax is added to fill up the space between the tube and rim of the opening in the bottom of the tray. The top of the overflow tube should now be about 1.5 cm. below the top of the tray.

10. *Sand tray.*—This piece of stoneware has a volume of about 6.5 liters.

11. *Quartz sand and magnetite.*—Five kilograms of sand is mixed with 25 grams of powdered magnetite, $FeO \cdot Fe_2O_3$. The layer of gravel in the bottom of the tray should be wetted before adding the sand. A sand, of which 50 per cent. is retained by a 60-mesh screen after passing a 40-mesh

[1] Supplied by B. F. Goodrich Company, size 3/16-inch inside diameter, with 1/8-inch wall thickness.

[2] A hard rubber tubing supplied by Kirkhill Rubber Company, Los Angeles, California, with 22/32-inch inside diameter and 25/32-inch outside diameter, also appears to be satisfactory in preliminary trials and it is somewhat less expensive.

screen, has been found to be satisfactory.[3] In solutions at pH 6, the magnetite has supplied ample iron to all plants tested (**1, 2, 3**).

12. *Air vents.*—Without these glass tubes which extend into the gravel, air is locked in the sand when the trays are flushed, thus preventing a free, uniform, downward movement of solution.

13. *Quartz gravel.*—One kilogram of gravel of a size which passes a 4-mesh screen and is held on an 8-mesh screen is suitable. The sand does not work down into the gravel if the latter is wetted before filling.

14. *Asphalt compound.*—This product, which is sold under the trade name G–K sewer-joint compound, has a rather low melting point, but it maintains a good union with the glazed walls of the sand tray when it cools.[4]

15. *Porous disc.*—These are purchased in 6¼-inch squares 1 inch thick. A 30-mm. hole is first bored through the center on a press drill with a steel tube. From 10 to 14 of them are then strung on a 30-mm. rod, clamped into a lathe, and then turned down to a diameter of 6⅛ inches. The material works easily. The permeability of the discs is about 600 ml. per minute when the trays are filled with sand and the solution is at the height of the overflow.

16. *Red sealing wax.*—This is used to seal the overflow tubes, 9, into the bottoms of the sand trays. Five grams of finely powdered sealing wax added to a liter of culture solution has been found to be somewhat toxic to tomato plants in water cultures. The small solid piece used in this seal has shown no ill effects.

17. *Reservoir.*—The jars shown have a capacity of 14.5 liters. They have three outlets, each of which takes a no. 2 rubber stopper. The opening at 19 is set 4 inches to the right of those at 20 and 21.

18. *Cap for water gauge.*—This is a piece of ½-inch aluminum tubing with one end closed. Its use prevents the growth of algae in the gauge.

19. *Water-addition opening.*—In replenishing solutions, this stopper is removed and the point of a self-closing radiator faucet inserted.

20. *Water gauge.*—This is a 5- or 6-mm. glass tube bent in the shape shown and painted black below cap.

21. *Outlet for emptying reservoirs.*

22. *Culture solution.*—Salts for 13 liters are used in making up 11 liters of solution. Two liters of water are then added to the surface of the sand and when draining has stopped, a wax mark is made on the water level gauge to indicate the initial volume. The five kilograms of sand retain between 1.6 and 1.8 liters of solution against gravity.

[3] This sand is supplied by the Industrial Sands Division, Corona, California.

[4] Atlas Mineral Products Company, The Deming Company, 4227 Whiteside Avenue, Los Angeles, California.

23. *Magnetic air valve.*[5]

24. *Air pressure line.*—Air pressures up to about 50 pounds are satisfactory. Where a large number of cultures are operated from a single air line, the latter pressure beyond the pressure regulator is desirable. A discharge rate of 0.7 cu. ft. of air per minute per culture gives a satisfactory solution delivery rate.

25. *Time switch.*—The General Electric TSA–14 time switch can be used alone without time-switch 26 if the cultures are flushed each hour throughout the 24 hour period. This timer makes a complete cycle every 60 minutes. The duration of the "on" period can be set for any fraction of the 60-minute period. Three minutes are ample for the complete replacement of the solution held by the sand, providing air pressures are maintained.

26. *Time switch.*[6]—This switch is connected in series with switch 25 in such a way that the circuit to the magnetic valve is completed only during an "on" period of both timers. The "on" and "off" pins on the 24-hour dial of the Sangamo timer cannot be inserted nearer together than 15 minutes. This switch is used to limit the flushing of the sand trays to selected hours.

Water cultures

In using the sand-culture solution reservoirs as water cultures, the air hose leading from each greenhouse bench is disconnected from the non-continuous high-pressure air line and attached to a continuous low pressure outlet. Carbon-tube aerators are employed following a suggestion originating with P. R. Stout and D. I. Arnon of the University of California.

1 and 4. *Glazed stoneware cover.*—Two sizes of tapered holes are provided in this cover for mounting seedlings of various sized plants. The cotton mountings for seedlings stay in place better if the holes are not glazed.

2. *Rubber or Koroseal connection.*

3. *Glass or ¼-inch aluminum tubing.*

5. *Aerator.*—Plain carbon pipe,[7] diameter inside ½-inch, outside ¾-inch. Five pounds of air pressure can be used without blowing the cork stoppers if they are inserted after having been dipped in hot G–K Compound.

Stoneware

The stoneware described is manufactured[8] in accordance with specifications. Plain solid covers to fit trays and reservoirs of this size are available at nominal cost.

[5] Made by Magnetic Controls.

[6] Sangamo Type TC–11 (sold by Graybar Electrical Co.).

[7] Supplied by National Carbon Company, Inc.

[8] By the Pacific Clay Products, 306 West Avenue 26, Los Angeles, California. Reservoirs (U. S. 3), $1.00; sand trays (U. S. 4), 60 cents; water-culture covers (U. S. 5) 50 cents; and sand-tray covers (U. S. 6), 60 cents.

The squares of insulating brick were cut to order (1 inch by 6¼ inches).[9] There are a number of insulating bricks of similar composition and volume weight on the market, but the Vitrefrax has been the only one so far tested that had a high permeability.

The other pieces of the equipment are all standard products and the prices would accordingly vary with location and purchasing conditions.

Large out-of-doors sand cultures for annual and tree crops

The new sand cultures for annual crops grown to maturity, figure 4, have dimensions similar to the one previously described (3, fig. 2) except that the

FIG. 4. Out-of-doors sand cultures for annual crops.

depth of the reservoir has been increased from 18 to 29 inches, giving a capacity of 2,400 liters. Asphalt painted transite in pieces ¼-inch thick are used in place of tin to suppress the growth of algae. The motor pump is placed in a housing at the end of the bed below the upper level of the solution. Aluminum silicate brick are used for drainage in place of gravel. The ends of 39 standard size brick (2 × 4.5 × 9 inches) are bevelled so that they rest on the correspondingly bevelled edges of the concrete cross tile. The walls of these unit type beds are 6 inches thick, thus permitting the use of a recess rather than angle iron to support the transite.

[9] Furnished by the Vitrefrax Corporation, 5050 Pacific Blvd., Los Angeles, California, in lots of 100 at 35 cents each.

The 18 deep sand beds for tree crops at the U. S. Regional Salinity Laboratory, Riverside, California, have inside dimensions of 5 × 10 × 6 feet deep. They are spaced 10 × 18 feet center to center. Whole permeable brick are laid above drainage ways cast in the concrete floor. A number of tubes for the escape of air during flushing are extended into the gravel laid above the permeable brick. The inside dimensions of the reservoirs are 7 × 4 × 6 feet deep.

A high silica cement-lined pipe sold under the trade name "Duroline" is used for all plumbing with promising results from the standpoint of corrosion.

The concrete walls of these cultures are made nearly impermeable and chemically inert by a two-coat sprayed-on dressing of emulsified asphalt.[10]

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[10] The material used is sold under the name "Static Protective Coating" by The Flintkote Company.

ANTAGONISTIC ACTION OF CHLORIDES ON THE TOXICITY OF IODIDES TO CORN[1]

J. C. LEWIS AND W. L. POWERS

During recent investigations of the relation of iodine to plant nutrition, we found iodides to exert toxic effects on corn at very much lower concentrations than had previously been reported by MAZÉ (5). MAZÉ's nutrient solutions contained 22 p.p.m. of added chlorides. Our solutions contained only such concentrations of chlorides as were carried as impurities by the salts and water used in their preparation. We therefore conducted experiments to determine whether chlorides antagonize the toxic effect of iodides on corn.

Methods

Corn seeds were germinated in the basal nutrient solution described below. When 3 to 4 inches high, uniform seedlings were transferred to 4-gallon stoneware jars containing the basal nutrient solution. Iodide and chloride additions were made 2 to 3 days later.

The basal nutrient solution used had the following composition: calcium nitrate, 0.007 M; magnesium sulphate, 0.005 M; monopotassium phosphate, 0.005 M; dipotassium phosphate, 0.0015 M; boron as boric acid, 0.5 p.p.m.; manganese as sulphate, 0.5 p.p.m.; zinc as sulphate, 0.05 p.p.m.; copper as sulphate, 0.02 p.p.m.; and molybdenum as ammonium molybdate, 0.05 p.p.m.

Tap water was used in the preparation of this solution. Ferric tartrate was added twice weekly at the rate of 1 ml. of a 0.5 per cent. solution per liter of nutrient solution. In one experiment, humate iron (3) equivalent to 2 p.p.m. of iron was used in place of ferric tartrate. The initial pH of the basal nutrient solution was approximately 5.8. The pH of the culture solutions was adjusted every 2 weeks, or as needed, while the solutions were renewed at 30-day intervals.

Iodine was added as potassium iodide. Chlorides were added as a mixture of the calcium, magnesium, and potassium salts, in the same cation proportions as these cations appeared in the basal formula.

The methods used for iodine analyses varied with the anticipated iodine content. High-iodine samples were fused with potassium hydroxide. Low-iodine samples were burned in the VON KOLNITZ-REMINGTON (7) oxygen torch. The iodine was then recovered by the distillation method of BRATTON and MCCLENDON (2), followed by bromine oxidation and thiosulphate titration.

[1] Published as Technical Paper no. 362 with the approval of the Director of the Oregon Agricultural Experiment Station as a contribution of the Soils Department.

Chlorine was determined by the A.O.A.C. (1) gravimetric method after a nitrous acid treatment (6) designed to remove iodine.

Results

The results of our first experiments to determine the toxic levels of iodides for corn are given in table I. The growth period was 60 days, by

TABLE I

EFFECT OF IODIDES ON GROWTH AND COMPOSITION

IODIDE ADDED TO SOLUTIONS	MEAN DRY WEIGHT OF TOPS	IODINE CONTENT OF TOPS	TOTAL IODINE IN TOPS	CHLORINE CONTENT OF TOPS	TOTAL CHLORINE IN TOPS
p.p.m.	*gm.*	*p.p.m.*	γ*	*p.p.m.*	*mg.*
0	17.2 ± 2.0†	0.05	0.9		
0.1	14.0 ± 1.8	8.6	120	1510	21.2
0.5	11.8 ± 1.8	58	680	1600	18.9
1	9.9 ± 1.5	88	870	1850	18.3
5	2.2 ± 0.4	439	950	7820	17.3

* The microgram (γ) is 0.001 mg.
† The standard error of the mean is used throughout this paper.

which time the corn had immature ears. Duplicate jars with 3 plants per jar were used for each treatment, except that in which 5 p.p.m. of iodine was added for which only 1 jar was used.

The data show growth repression even at 0.1 p.p.m. of added iodine, while 5 p.p.m. is extremely toxic. Chlorosis is pronounced in the presence of 5 p.p.m. of iodine. The iodine content of the tops is approximately proportional to the iodine concentration of the nutrient solutions. The chlorine content appears to be related to the iodine concentration of the nutrient solutions.

The next two experiments were conducted to determine the effect of 20 p.p.m. of added chlorides on the toxic action of 2 p.p.m. of iodine. In the first of these experiments, humate iron was used as a source of this element. These plants became uniformly chlorotic, and growth was greatly repressed. Local additions of iron salts on the leaves produced green spots, showing that the symptoms were caused by iron deficiency. These plants are referred to hereafter as "iron-deficient."

In the second experiment ferric tartrate was used as a source of iron. These plants, which did not suffer from iron deficiency, are referred to hereafter as "normal." After 30 days the plants in each jar were weighed and those 4 nearest the mean weight were returned to the culture solutions. After a total of 46 days these plants had reached the tasseling stage and were then harvested. Triplicate jars were used for each treatment. Eight seedlings per jar were used.

The results, summarized in table II, indicate that 2 p.p.m. of iodine reduced the yields by approximately 40 to 60 per cent. About one-half of this injury was preventable by the addition of 20 p.p.m. of chlorides.

TABLE II

EFFECT OF CHLORIDES ON THE TOXICITY OF IODIDES

Treatment added to solution	Iron-deficient corn	Normal corn	
	Green weights after 27 days	Green weights after 30 days	Green weights after 46 days
	gm.	*gm.*	*gm.*
Chloride 0 p.p.m. Iodide 0 p.p.m.	6.35 ± 0.37	27.0 ± 1.0	120 ± 8
Chloride 20 p.p.m. Iodide 0 p.p.m.	7.57 ± 0.34	28.5 ± 1.1	108 ± 5
Chloride 0 p.p.m. Iodide 2 p.p.m.	3.61 ± 0.17	16.3 ± 1.3	44 ± 5
Chloride 20 p.p.m. Iodide 2 p.p.m.	6.07 ± 0.29	21.7 ± 1.0	80 ± 9

Chlorine and iodine analyses of certain of the plant materials were made to see if the chlorides act by repressing the absorption of iodine. These data, presented in table III, indicate that the iodine content has not been especially affected by the chloride concentration of the solutions. The chloride content, in most cases, has been markedly increased by the presence of 2 p.p.m. of iodine in the cultural solutions.

TABLE III

EFFECTS OF IODIDE AND CHLORIDE TREATMENTS ON COMPOSITION OF CORN PLANTS

Treatment added to solutions	30 day-old plants		46 day-old plants					
	Tops	Roots	Leaves		Stems		Roots	
	Chlorine	Chlorine	Chlorine	Iodine	Chlorine	Iodine	Chlorine	Iodine
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Chloride 0 p.p.m. Iodide 0 p.p.m.	2940	2070	1410	0.46	1320	0.14	840	0.42
Chloride 20 p.p.m. Iodide 0 p.p.m.	5560	2980	5520	0.33	4750	0.14	2050	0.51
Chloride 0 p.p.m. Iodide 2 p.p.m.	4540	2650	2860	236	2460	166	1440	138
Chloride 20 p.p.m. Iodide 2 p.p.m.	7560	3490	5980	240	5630	156	1690	136

Tap water was used in these experiments since previous work had shown that nutrient solutions prepared from tap water and C. P. chemicals had iodine contents of the order of 0.001 p.p.m. Corvallis city tap water has had an average iodine content for the past several years of 0.00035 p.p.m.

After analyses were made of the plants grown in solutions to which chlorides had not been specifically added, it became apparent that the basal nutrient solution contained very appreciable amounts of chlorides. Subsequent analyses indicated a chloride content of about 3 p.p.m. in the tap water, while the chlorine contributions from the nutrient salts and the seeds were negligible.

Discussion

Under the experimental conditions the extent to which chlorides antidote the toxic action of an iodide concentration that gave a 50 per cent. reduction in yield was quite marked and fairly uniform. The magnitude of this degree of toxicity is well suited to studies of this type. The reduction in yield is marked enough to give clear-cut results without serious interference from secondary factors such as chlorosis. No work was done to determine protective Cl/I concentration ratios such as those worked out for other chemically-related elements by HURD-KARRER (4).

Since merely raising the chloride concentration in the nutrient solution from approximately 3 p.p.m. to 23 p.p.m. gave a substantial antidoting effect, one is led to speculate that the toxicity of a given level of iodides would have been greater had chlorides been more rigorously excluded from the culture solutions. Likewise, the response of iodide toxicity to added chlorides may depend on the amount of chlorides already present in the solutions and may be greater at lower basal chloride concentrations.

In table III it may be seen that the chlorine contents of the 30-day-old normal corn are considerable higher than those of the 46-day-old normal corn. Moreover, iodide treatment is almost as effective as chloride treatment in raising the chlorine content. The possibility of an artifact wherein iodides would be mistaken for chlorides in the analyses was eliminated by removal of iodine by nitrous acid (6) treatment. This separation was found by actual test to be complete under our conditions.

The effect of iodine in increasing the chlorine content may be most readily explained by assuming that the primary effect of the iodine is in inhibiting growth, while it has little effect on the absorption of chlorides. In this event the total chlorine absorbed per plant should be relatively constant. That this is the case may be seen in table I. Similar calculations for the other experiments are in agreement with these data.

In general, the iodine content of the plants is approximately proportional to the iodide concentration in the solutions. The absolute amount of iodine in the plants is therefore determined in part by the effect of iodine on

growth, as well as by the iodine content of the plants. The chloride concentration in the solutions apparently does not affect the iodine content of the plant tissues.

Since chlorides do not appear to act by repressing iodine absorption, they may act by replacing iodine in certain physiological reactions within the plant. This general reaction was proposed by HURD-KARRER (4) in her "mass-action antagonism" hypothesis. In this case, the increased chlorine content of the plant may be a protective response of the plant to iodide toxicity, the plant maintaining a similar chloride absorption rate per plant in spite of the reduced growth rate.

The apparent lack of relation between iron deficiency and the chloride-iodide antagonism is noteworthy. Extreme iodide toxicity (5 p.p.m. in table I) is superficially very similar to severe iron-deficiency chlorosis. Corn grown in solution cultures is very susceptible to iron deficiency. This may have contributed to some extent to the variability of the individual plant weights obtained in these experiments.

It is of interest to note that marine plants, and especially seaweed, normally have iodine contents that may be acquired by land plants only in the presence of highly toxic iodine concentrations. In the case of marine plants, the plentiful supply of chlorides in the environment may be of prime importance in permitting the accumulation of iodine without toxic effects.

Of more immediate importance is the fact that the discovery of the iodide-chloride antagonism permits a partial explanation of certain discordant results in the literature dealing with the effects of iodine on plant growth. The presence of an adequate concentration of chlorides, by reducing the toxic action of iodides, may profoundly influence the outcome of such experiments.

Summary

1. The addition of 20 p.p.m. of chlorides partially prevented the toxic action of 2 p.p.m. of iodides on corn in solution cultures.

2. The iodine content of the plant material was not affected by the chloride concentration in the solutions, but the chlorine content was increased markedly in the presence of 2 p.p.m. of iodides.

3. The iodide-chloride antagonism appeared to be independent of iron deficiency.

We desire to thank Dr. J. R. HAAG of the Department of Agricultural Chemistry for his kind cooperation in furnishing laboratory facilities for the iodine analyses.

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SOME COMMENTS ON THE MECHANISM OF PHLOEM TRANSPORT

T. G. MASON AND E. PHILLIS

In a recent paper, CRAFTS (**6**) points out that "Present theories fall into two categories: (1) movement of solute molecules taking place in, through, or upon the surface of sieve-tube protoplasm, and which results from protoplasmic activity; (2) mass flow of solution through sieve-tubes or phloem, related, at least indirectly, to activity of photosynthetic tissues, and not dependent upon the activity of the sieve-tube protoplasm." His paper presents his objections to the first mechanism and the case for mass flow.

"The pressure-flow theory," he now says, "pictures acceleration in the sieve-tube as resulting from a hydrostatic pressure, developed osmotically and exerted impartially upon solute and solvent." "Conduction," he thinks, "is permitted by a high permeability of the sieve-tube cytoplasm resulting from a decline in its activity state."

The prototype of pressure-flow theories is that advanced by MÜNCH (**16**). One prerequisite of this theory is open sieve-pores,[1] and provided that those are present, MASON, MASKELL, and PHILLIS (**12**) concluded that the observed gradients of osmotic pressure were adequate to produce the required rate of flow through the sieve-tubes. But all recent investigators are unanimous in concluding that the sieve-pores are not open. CRAFTS (**3**) stressed this fact and his recognition of it led him to suggest that the pressure stream travelled longitudinally through the walls. The fallacy in his calculation of wall resistance was exposed by STEWARD and PRIESTLEY (**22**).

CRAFTS (**6**) has since modified his position by postulating movement along the lumen of the sieve-tube and through the transverse walls separating sieve-tubes. The protoplasm of the functional sieve-tube, he believes, has lost the property of semi-permeability. Presumably there would also be some longitudinal movement through the walls of the phloem parenchyma and the sieve-tubes. His belief that the sieve-tubes are freely permeable is based on his inability to plasmolyze these elements. He seems to think that the sieve-tubes become senescent before the other phloem elements. In a previous paper (**14**), we have pointed out that sieve-tubes may be plasmolyzed provided they are not injured. CURTIS and ASAI (**7**) have very recently confirmed our observations. There would thus appear to be no experimental grounds for CRAFTS's belief that the sieve-tubes are freely per-

[1] The vacuoles of adjacent sieve-tubes were assumed to be continuous through the sieve-pores.

meable.[2] Apparently he has not realized that the sieve-pores allow pressure to be transmitted from one element to another; thus sieve-tubes might be injured[3] at some distance from a cut. As to the senility and low activity of the sieve-tube, SMALL (**21**) has recently demonstrated that protoplasmic streaming occurs in these elements. One wonders whether the sieve-tubes are always as short-lived as CRAFTS believes. In palms, for instance, it would appear that they often live for a great many years.

CURTIS and ASAI (**7**) have also pointed out that if the sieve-tubes are permeable to their contents there would be lateral leakage since there is nothing equivalent to the Casparian strip in the stem. They found that "the phloem exudates from Fraxinus and that from Cucurbita will strongly plasmolyze their respective phloem parenchyma cells" and point out that this "demonstrates that the sieve-tube membranes cannot be completely permeable to their contents." A further point is that hydrostatic pressure could not be generated in the fine veins of the leaf (**17**) if the transition cells were freely permeable.

Even if the sieve-tube could not be plasmolyzed, it would only indicate (apart from the possibility that it had been killed) that its protoplasm was equally permeable to water and solutes. It would not follow that its permeability to water had been affected. CRAFTS's modification of the MÜNCH theory involves movement through highly permeable protoplasm instead of movement through an open channel. The observed pressure gradients were only just sufficient to account for movement through open sieve-pores and not through sieve-pores filled with a viscous colloid, which is what CRAFTS's suggestion amounts to. Actually the capacity of the phloem to conduct water appears to be extremely limited. Thus, when the wood is removed from the stem of a cotton plant between the foliage region and the root, the leaves rapidly wilt. Furthermore, when all the leaves are removed, the unlignified upper part of the stem fails to remain turgid. The observations of DÖPP (**9**), who has shown that the growth of young fruits of apple is completely inhibited if the wood leading to them is broken, point in the same direction.

We now enter territory that is still somewhat debatable. The pressure-flow theory requires a hydrostatic pressure gradient in the conducting tracts. It has been generally assumed that this gradient is caused by differences in osmotic pressure. Osmotic pressure gradients of 2 to 9 atmospheres per meter in the sap exuded from sieve-tubes have been demonstrated by DIXON (**8**). BENNET-CLARK, GREENWOOD and BARKER (**1**) have recently

[2] It is of interest to note that the cells of the bast-glomeruli of the yam (**10**) can be readily plasmolyzed.

[3] In a recent paper (**19**) we have considered some of the factors responsible for the production of sap from protoplasm as a result of pressure injury.

suggested that the turgor pressure of the cell is only partly due to the osmotic pressure of the vacuole. They postulate in addition a secretion pressure driving water into the vacuole. Our own observations on the osmotic pressure of the vacuole (**15**) have led us to suggest that the contribution made by the osmotic pressure is very small compared with that of the secretion pressure. Our suggestion has since received confirmation from the very recent work of BENNET-CLARK and BEXON (**2**). It would thus appear that the whole question of the existence of hydrostatic pressure gradients in the sieve-tube system is in doubt.

In commenting on what he terms "the inadequacy of mass-tissue analysis for studies on translocation," CRAFTS (**6**) says "mass analysis gives predominantly a picture of vacuolar concentrations and indicates simply the amount of solutes maintained in the vacuoles." We have recently shown (**15**) that the concentration of solutes in the vacuole is only a small fraction of that in the protoplasm. Our results have since been confirmed by BENNET-CLARK and BEXON (**2**). We were also able to show that the protoplasm normally occupies the bulk of the cell. Mass analysis therefore gives predominantly a picture of the concentration in the protoplasm and is accordingly justified in studies on translocation. Thus, in the leaf, over 90 per cent. of the sugar may be in solution in the protoplasm.

CRAFTS remarks that "continued experiments on chilling and coating petioles have failed to prove that sieve-tube protoplasm plays an essential rôle in longitudinal transport." But we have shown (**13**) that withholding the supply of oxygen to the stem results in a stoppage of transport only as long as oxygen is withheld, and that reducing the supply of oxygen to defoliated fruiting branches retards the rate of carbohydrate transport to the boll. CRAFTS apparently believes that these results may have been caused by altered permeability. It will be evident that this explanation is in direct conflict with his statement that such "experiments have failed to prove that sieve-tube protoplasm plays an essential rôle in longitudinal transport."

CRAFTS (**5**) is now sensible that mere exudation of sap is not evidence of a directed mass flow of solution through the sieve-tube system (**22**). He has accordingly attempted to show that the exudate is not of local origin. He claims that on cutting away 36 cm. of stem from a squash plant the volume of the exudate was equal to the volume of phloem in 173 cm. of stem. The stem was cut repeatedly in thin slices. He remarks that "during the collection several internodes were removed." He says nothing about the leaves and petioles on the nodes. Until it is clear how much sap was contributed from this source, his claim that the exudate is not of local origin must remain in suspense. His belief that the exudate depends on the activity of the photosynthetic tissues would appear to be without foundation, for

we have found that active exudation proceeds from pumpkin stems a number of days after the leaves had been removed, while he himself (**4**) found that "the exudation from the cut peduncle of a fruit stored for six weeks was normal." As far as we are aware it has not yet been demonstrated that the direction and rate of transport are in any way related to the direction and rate of exudation (**11**).

The calculations of MASON and MASKELL (**11**) led them to conclude that the diffusion constant of sugar in the phloem is almost forty thousand times as great as the diffusion constant in a 2 per cent. solution of sucrose in water. CRAFTS (**6**) points out that these rates are based on the whole area of the phloem, including sieve-tubes, companion cells, parenchyma, and the cell walls of these cells and are therefore much too small. He concludes that sugars must move in the sieve-tubes at rates at least 200 times those originally proposed by MASON and MASKELL. While he may be correct in concluding that MASON and MASKELL's calculations are too low, it is very doubtful if they ought to be raised as much as he suggests. He claims to have measured, for instance, the thickness of the cytoplasmic layer of the sieve-tube, even though SINNOTT and TROMBETTA (**20**) concluded that "the problem of determining cytoplasmic volume in plant cells seems almost a hopeless one." We have referred in a recent paper (**15**) to the difficulties involved in measuring protoplasmic volumes. Without going through the whole of his calculations we should refer to his failure to make any correction for the collapse of the sieve-tube when sections are cut. On the other hand, the figures of MASON and MASKELL may be too high for two reasons. In the first place, the sugar gradients in the sieve-tubes may be steeper than in the phloem parenchyma. Secondly, SMALL (**21**) has demonstrated protoplasmic streaming in mature sieve-tubes. While streaming alone could not account for the acceleration of diffusion at the rates calculated by MASON and MASKELL, the existence of streaming would greatly reduce the diffusion constants required to move carbohydrates at the observed rates. The point to stress in connection with the calculations of MASON and MASKELL of the diffusion constant for sugar in the sieve-tube is that it is immeasurably greater than the diffusion constant of sugar in water.

To those who believe that protoplasm has a gel structure consisting of a predominantly protein reticulum and an aqueous continuous phase, such diffusion rates will be incomprehensible. Protoplasm, however, has almost certainly not a typical gel structure. However this may be, it has structure, and structure that exists only as long as metabolic energy is supplied. Energy is utilized in maintaining structure and, in virtue of this structure, protoplasm has properties quite unlike those of water. Moreover, the amount of energy required to maintain this structure would bear no relation to the amount of energy that would be required to accelerate diffusion

in water. The hypothesis of *activated diffusion*, in short, regards protoplasm as an *activated* liquid with diffusion constants and solubilities (**18**) quite different from those of water. Until the physicist knows more concerning the nature of liquids (*e.g.*, liquid helium) and of activated diffusion, and the biologist knows more about the physics of protoplasm, it is impossible to say how the diffusion of solutes is accelerated in the sieve-tube.

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pH DETERMINATION IN PLANT TISSUE

A. R. C. Haas

In a recent investigation[1] a procedure was found that permitted fairly accurate determinations of the pH of soils of low moisture percentages. One of the essentials of the method was the use of a horn spoon in spreading the moisture or soil films uniformly throughout the rather dry soil mass.

When plant tissue is finely cut in a grinder, relatively few of the cells are actually opened. The important fact is that the contents of the cut cells is smeared over the uncut groups of cells. On account of the somewhat analogous nature of the state of the soil and of the finely divided plant tissue, it appeared highly probable that the same methods that were applied in the determination of the pH of soils could be applied equally as well in the pH determination of plant tissue.

Materials and methods

The pulp of date fruits, the peel of lemon fruits, and the leaves of avocado seedlings furnished the material for the tests. A Beckman pH meter (model g) with a shielded glass electrode, and ten-foot extension cables attached to both electrodes, served as the means of measuring the pH.

The date fruits were wiped clean without being previously washed. The lemons and the avocado leaves were washed in running distilled water and then wiped dry. The date fruits were cut with a knife in order to discard the seed. The lemon fruits were peeled and the pulp was discarded, care being taken in the peeling process to avoid piercing the bounding membranes of the acid pulp. The samples were finely cut by means of a Universal food chopper, no. 3, making use of the finest of the four cutters. The chopper was used in place of the Wiley mill because of the relative ease in the washing and drying of the chopper, although most any cutting machine may be used. With very woody material the mill is most useful, although pencil sharpeners or whittling with a knife may be usefully employed.

The pH was determined immediately after each lot of material was cut in the chopper. The same sample of finely cut plant tissue that was used in the determination of pH was at once placed in a wide-mouthed glass jar that was tightly closed by means of a glass cover with suitable rubber gasket and metal clamp. As each sample was finished it was temporarily stored in a refrigerator until the group of samples was completed. Refrigeration was then obtained by storing the jars overnight in an underground freezing room of a cold storage company. The following day the jars were taken to the

[1] Haas, A. R. C. The pH of soils of low moisture content. Soil Science (In press).

laboratory where, after thawing the frozen plant tissue, the juices were expressed and the pH values determined in the extracted juices. Twenty thousand pounds pressure was used for the sap extraction from the date pulp and avocado leaves, while fifteen thousand pounds was used for the sap extraction from the lemon peel.

After the plant tissue was finely cut with the chopper it was thoroughly mixed in a shallow, wide glass dish. The juice from the cut cells was uniformly spread throughout the mass by means of a horn spoon which was used in the same manner as a spatula is used in mixing ointments. Heating of the material was overcome by working rapidly and by spreading out the material immediately after it emerged from the chopper. With some materials brief refrigeration may be of assistance in this regard. The cut tissue is placed in a beaker which is tapped on a folded towel on the table top. With the hand or any suitable tool, the tissue is well compacted within the beaker and the electrodes and thermometer are quickly inserted.

Both electrodes were held in an electrode holder provided with a spring clamp on a metal support. Upon squeezing the clamp the electrodes could be raised or lowered. The electrodes were pressed into the cut tissue and the surface tissue was firmly compacted about the electrodes in order to cover the unshielded portion of the glass electrode. A sufficient depth of well compacted tissue should occur between the bottom of the beaker and the sensitive part of the glass electrode. This material acts as a bumper or buffer in preventing the glass electrode from being broken against the bottom of the beaker. These electrodes are supposed to withstand pressures of thirty-five pounds and probably will withstand much higher pressures. In the inserting of the glass electrode into soils,[1] whenever care was taken to avoid contacting the bottom of the beaker, these electrodes withstood any slow steady pressures that could be applied with the hands.

After the temperature adjustment of the pH meter was made, the pH readings of the tissue were made. The electrodes then were more firmly pressed into the tissue and the surface tissue again was tightly compacted about the electrodes. Thus, pH readings were made a second time. This repetition or renewal of the electrode pressures and the surface compacting about the electrodes with the accompanying pH readings was continued until three successive repetitions of the process showed no appreciable change in the pH readings. This insures that the contact between the juice films on the tissue and the glass electrode is the most intimate that is obtainable.[1]

The sample of tissue was then removed as described and after being frozen, the sap was extracted under pressure.[1] The pH of the juice was then determined and compared with that of the finely divided and freshly cut, but unfrozen, plant tissue.

Results

pH OF CUT FRESH FRUIT TISSUE AS COMPARED WITH THAT OF THE JUICE OF THE FROZEN TISSUE

A total of 100 unripe green dates were collected at Indio on July 24, 1939; the pulp weighed 158 grams and the seed 19 grams. The peel of seven ripe healthy lemons was used in the test with lemons. Table I shows the agreement between the pH values of finely cut fresh fruit tissue and those of the sap extracted from these tissues after being frozen. With date pulp which is very high in sugars there should be considerable opportunity for chemical changes to take place after the pulp is cut and yet the results were most promising, even in these preliminary tests.

TABLE I

pH OF FRESHLY CUT PLANT TISSUE AND OF THE SAP OBTAINED FROM THE FROZEN TISSUE

Sample no.	Finely cut fresh plant tissue	Juice extracted from frozen plant tissue
	Deglet Noor date pulp	
	pH	*pH*
1	5.55	5.45
2	5.65	5.47
3	5.55	5.41
4	5.60	5.50
5	5.62	5.50
Distilled water	5.27	
	Peel of ripe healthy lemons	
1	5.30	5.31

pH OF FINELY CUT FRESH AVOCADO LEAVES AND OF THE JUICE EXTRACTED FROM THE TISSUE AFTER FREEZING

This experiment should not only serve to reveal whether the pH values of cut fresh tissue differ from those of the sap of the same sample of tissue after freezing, but should also indicate the effect, if any, of the pH of the soil upon that of the sap in plant leaves.

Hanford soil (pasture soil), obtained near the Citrus Experiment Station, was used in these cultures. An avocado seed was planted in each container of soil. The containers were six inches in diameter by seven inches high and contained 4000 grams of air-dry soil. Distilled water was used at all times. The cultures each received a total of 1.0947 grams of nitrogen applied in the form of calcium nitrate solution. This nutrient was divided into three equal applications to the soil during the growth of the cultures from May 1, 1939, to June 5, 1940. Various amounts of sulphur (table II) were applied to the surface of the soil after the tops were several inches high.

TABLE II

RESULT OF COMPARATIVE METHODS OF DETERMINING THE pH OF AVOCADO LEAF SAP OF PLANTS GROWN IN SOILS OF DIFFERENT pH

SULPHUR ADDED TO THE SURFACE OF SOIL CULTURES	SOIL SAMPLES AT END OF THE EXPERIMENT		MOISTURE PERCENTAGE OF THE SOIL AT END OF THE EXPERIMENT	FRESH WEIGHT OF LEAVES	FRESH WEIGHT OF TRUNK	FRESHLY CUT AVOCADO LEAVES	JUICE EXTRACTED FROM FROZEN AVOCADO LEAVES
	pH AT THE CULTURE SOIL MOISTURE PERCENTAGE	pH OF SUSPENSION OF OVEN-DRIED SOIL 1-5 SOIL-WATER RATIO					
gm.	*pH*	*pH*	%	*gm.*	*gm.*	*pH*	*pH*
0	6.42	6.86	10.1	55	64	5.45	5.47
0.05	6.53	6.72	10.3	85	101	5.47	5.50
0.10	6.49	6.84	11.8	69	90	5.41	5.44
0.50	5.90	6.37	11.0	86	132	5.40	5.40
1.00	5.26	5.95	8.2	88	106	5.43	5.42
1.50	4.82	5.42	6.9	98	104	5.55	5.57
2.00	4.72	5.04	9.1	91	104	5.40	5.37
2.50	4.39	4.83	6.8	89	99	5.63	5.70
3.00	4.21	4.55	6.6	98	102	5.80	5.98
3.50	4.31	4.46	6.8	65	77	5.63	5.61
4.00	4.02	4.24	10.6	16	36	5.65	5.76
4.50	3.80	4.10	10.6	Leaves dead	30		
5.00	3.75	4.11	11.7	Leaves dead	31		
	Soil samples divided into upper and lower halves						
0	6.10	6.70	8.0				
	7.27	7.38	8.1				
0.05	5.85	6.60	5.9				
	6.70	7.27	5.8				
0.10	6.05	6.66	7.8				
	6.82	7.24	8.3				
2.00	5.06	5.31	4.5				
	5.14	5.20	5.4				

Soil samples were obtained when the experiment was terminated. Six cores of soil, taken the full depth of the container, were used for the pH determination at the culture soil-moisture content. When oven dried, these soil samples were used to determine the culture soil-moisture percentage at the time of sampling and were also used for the pH determination of soil suspensions at the 1 to 5 soil-water ratio. A few days after the soil sampling, additional samples in a few containers were taken at the upper and the lower three-inch depths.

Table II shows the pH values of the soil when the experiment was concluded. In every case the pH values obtained in suspensions at the 1 to 5 soil-water ratio exceed those determined at the culture soil-moisture percent-

age occurring at the termination of the experiment. The pH at this latter moisture content more nearly represents the pH that affects the growth of these plants than does the pH at the 1 to 5 soil-water ratio.

In the soil samples (table II) taken from the upper portion of the soil mass, the pH at the culture soil-moisture percentage was less than that of the lower or deeper portion of the soil mass. Any acidity produced by oxidation of the sulphur or by the use of the distilled water (pH 5.30) brings acidity to the upper portion of soil before that to the lower or deeper portion.

Where no sulphur was applied the growth was poor. Even the smallest application of sulphur greatly improved the growth. With a sulphur application above 3 grams, growth was retarded, while at the 4.5- to 5-gram application, the leaves were dead and remained attached to the trunk which was alive.

Table II shows the range of soil acidity at the termination of the experiment. At or below pH 4.00 the growth was seriously retarded. The pH values for the leaves by the two methods of preparing the tissue agree very well. It is of interest that at the pH values of the soil (determined at the culture soil-moisture percentage) above pH 4.50, the pH values of the leaf tissue or juice are all approximately the same. Below this soil pH value, the pH readings for the tissue, or its juice, show a very slight tendency to be higher. Why the reaction of the tissues or their juices should tend to be less acid while the reaction of the soils are more acid, will require further study. At any rate, at the pH values which are comparable to those already found in orchard soils, the pH of the leaf tissue is notably constant.

In tables I and II it will be seen that the pH of the leaf or fruit tissue experimented with, was close to 5.5. Other pH determinations, for example those of the juice of the pulp of citrus fruits, are very much lower than this. With tissues other than these, as for example in the tissues of roots, very low pH values were also encountered. These pH determinations were made by whittling the root into many shavings which were treated in the manner described for cut fresh tissue. This technique should prove most useful for tissues from which it is difficult to obtain much juice, such as woody twigs, and for those tissues in which high air-suction or pressures are being avoided.

Summary

The method used for the pH determination in soils of low-moisture content was found to be equally applicable to plant tissue. The agreement between results obtained in this manner and those obtained with juice extracted from frozen tissue was most satisfactory. Over a wide range of pH in the soil of avocado cultures, no significant change in the pH of the leaf juice was noted.

CITRUS EXPERIMENT STATION
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POTASSIUM DETERMINATION BY THE COBALTINITRITE METHOD AS AFFECTED BY TEMPERATURE AND pH

LELAND BURKHART

(WITH TWO FIGURES)

Rapid chemical analysis of plant and soil extracts for plant nutrients is being given widespread attention. Potassium is one of the most important and expensive plant nutrients and requires, therefore, special consideration of methods employed for determining its concentrations in soils and plant tissues.

The cobaltinitrite method for potassium determinations in extracts of soils and plants is becoming universal; many investigators, however, have not considered the importance of controlling temperature and pH during the precipitation reaction.

Early investigators noted the sparing solubility of the double salt prepared by precipitation of cobalt salts with strong solutions of potassium and sodium nitrite which led to the development of DE KONINCK's reagent as a qualitative test for potassium. ADIE and WOOD (**1**) in gravimetric and volumetric determinations of potassium in fertilizers and soils by the cobaltinitrite method found it necessary to have relatively high concentrations of potassium (0.5–1 per cent.) in the precipitating medium in order to obtain reliable results. The precipitation of potassium salts in the sodium cobaltinitrite is appreciably more sensitive on addition of silver salts but this depends on the solution being halogen-free (**2**). Lithium, thallium, and ammonium salts must not be present as these also give crystalline precipitates with sodium cobaltinitrite. SCHUELER and THOMAS (**3**) report that it is necessary to keep the precipitating medium cooled to 5° to 8° C. for several hours in order to obtain satisfactory results.

In the course of developing a satisfactorily sensitive method for the rapid determination of potassium in extracts employing sodium cobaltinitrite, it was necessary to determine the effect of temperature over a wide range of potassium concentrations in order to establish a satisfactory set of conditions for the precipitation reaction. Potassium standards were prepared from potassium chloride. All solutions used were cooled in water baths to the respective temperatures indicated in figure 1 before adding the dry sodium cobaltinitrite. In preliminary trials it was found that sodium cobaltinitrite in the dry form is more sensitive than saturated solutions in equivalent amounts, even when the latter are accompanied by higher concentrations of sodium attained by addition of salts such as sodium nitrate and sodium chloride. Sodium cobaltinitrite in the dry form was used at a

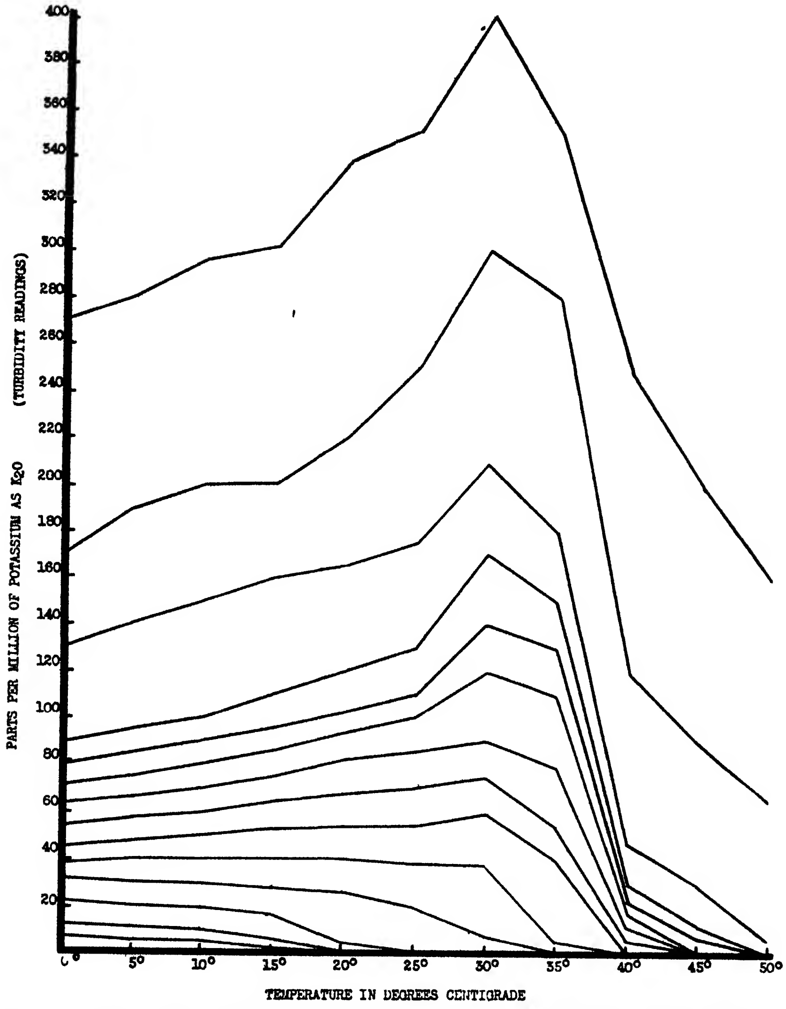

FIG. 1. Temperature of precipitating medium as affecting cobaltinitrite precipitation of potassium from prepared standards of potassium chloride. Turbidity readings at 10° C. used as standard of comparison.

rate to exceed 150 times the amount of potassium present in the precipitating medium.

To a pre-cooled 5-ml. portion of a potassium standard in a test tube there was added 60 mg. of sodium cobaltinitrite. Additional amounts of the reagent were used, however, at the rate of 60 mg. per 0.5 mg. of potassium when the concentration of potassium exceeded 100 p.p.m. After shaking the contents, 5 ml. of pre-cooled 95 per cent. ethyl alcohol were added and again shaken. One-half hour was allowed for the precipitation

and the turbidity compared with respective concentrations of potassium precipitated at 10° C. The turbidity developed by precipitation of various concentrations of potassium at 10° C. was used as a standard of comparison.

The results of the precipitations of the various concentrations of potassium at the respective temperatures are given in graphic form in figure 1. Of special significance is the fact that at low concentrations of potassium (5 to 20 p.p.m.) no precipitate is formed at or above temperatures of 20° C. Many of the extracts of productive peanut soils in North Carolina have concentrations of potassium which are less than 20 p.p.m. and it is, therefore, imperative that a low precipitating temperature be maintained when estimating low concentrations of potassium. At concentrations higher than 40 p.p.m. more precipitation occurs at 25° to 30° than at 10° C. Temperatures above 30° C. result in marked decreases in precipitation at all concentrations of potassium. It was found that the precipitation at 10° C. results in a much wider readable range of potassium concentrations. It is evident

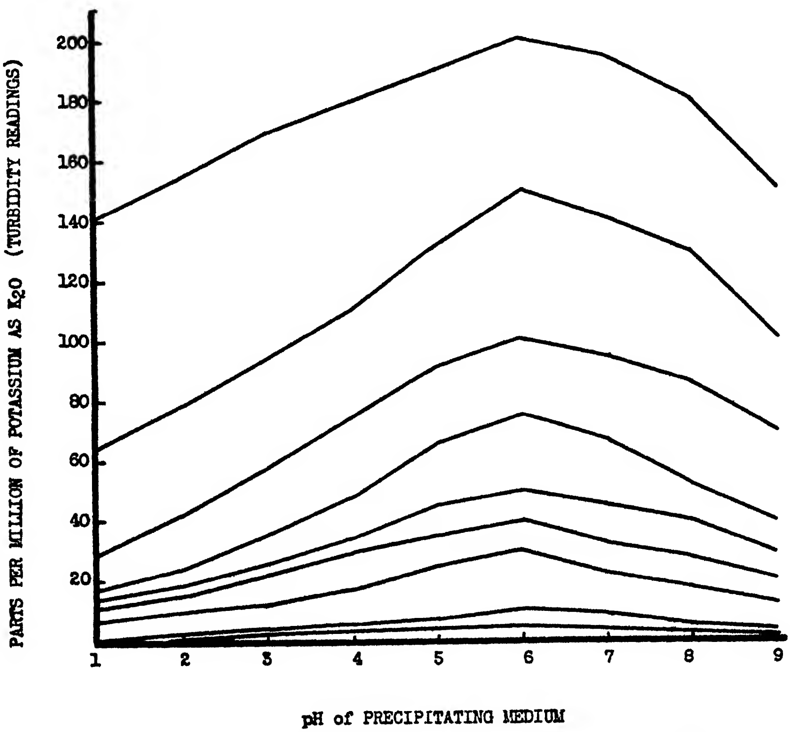

FIG. 2. Hydrogen ion concentration of precipitating medium as affecting cobaltinitrite precipitation of potassium at 10° C. from prepared standards of potassium chloride made up in respective buffer systems. Turbidity readings at pH 6 used as standard of comparison.

that the rapid determination of potassium by turbidity readings of cobaltinitrite necessitates controlling the temperature of the precipitating medium.

The influence of the hydrogen-ion concentration of the precipitating medium is shown graphically in figure 2. The respective pH buffer systems employed were as follows: pH 1 and 2, NaCl + HCl; pH 3 to 6, AcOH + NaOH; pH 7, combination of pH 6 and 8; pH 8 to 12, H_3BO_3 + NaCl + NaOH. These systems were made up from normal solutions to the desired pH by the electrometric titration procedure employing the glass electrode and a potentiometric pH meter. The phosphate buffer system could not be used because of interference of high concentrations of phosphate with the cobaltinitrite precipitation. As shown in figure 2 a buffer system at pH 6 is the most sensitive, therefore desirable as a precipitating medium for the estimation of all readable concentrations of potassium. Under alkaline conditions above pH 9 decomposition of the cobaltinitrite makes it impossible to determine potassium by this method.

From these results it appears that the precipitating medium should be maintained at 10° C. and pH 6 for the rapid determination of potassium by the cobaltinitrite method. Potassium standards should be prepared in buffer systems at the same pH as the extracts being examined. Under these controlled conditions of temperature and pH, a readable concentration range of 2 to 200 p.p.m. of potassium is attained. Turbidity measurements are satisfactorily made by the photoelectric method when using the proper color filter.

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CHANGES IN CHEMICAL COMPOSITION OF TWIGS AND BUDS OF YELLOW POPLAR DURING THE DORMANT PERIOD

J. JOSEPH McDERMOTT

(WITH TWO FIGURES)

Introduction

In order to study the chemical changes that accompany the breaking of dormancy in yellow poplar, *Liriodendron tulipifera* L., upon treatment with ethylene chlorohydrin vapor, it was necessary to determine what changes occur under natural conditions. It is with these latter changes that this paper is concerned.

Methods

During the dormant period a series of five samples of the buds and of the preceding year's growth was taken from a number of dominant and codominant yellow poplar trees. The samples were dried in an oven at 65° C., and were then ground in a ball mill until the material would pass through a 100-mesh sieve. Duplicate samples of from 25 to 50 grams were taken from this large sample, covered with 80 per cent. (by volume) aldehyde-free ethyl alcohol, and allowed to stand overnight. The liquid was decanted off and the last portion filtered through a hardened filter paper. The residue was dried at 105° C., coarsely pulverized, and extracted in a Soxhlet extractor until the percolate was colorless. The two extracts were united and made up to 1 liter with 80 per cent. alcohol. The residue from the last extraction was dried, coarsely ground, and preserved for analysis.

Reducing sugars were determined by the Shaffer-Hartman method in a 200-ml. aliquot of the extract. Total sugars were determined in the reducing sugar aliquot after hydrolysis with HCl at 73° C. for ten minutes.

Total soluble nitrogen was determined in 50-ml. aliquots of the extract, and total insoluble nitrogen on 1.0-gram samples of the residue by a modification of the Kjeldahl method.

A 1.0-gram sample of the residue was refluxed with 5 per cent. HCl for four hours, and the reducing value of the solution (as glucose) was reported as total reserve polysaccharides. Starch was determined by incubation at 38° C. of 2.0-gram samples of the residue suspended in 200 ml. of water with 0.1 gram of taka-diastase. Reducing sugars were determined on the solution.

Results

The analytical data obtained are assembled in table I and are graphically shown in figures 1 and 2. In explanation it should be stated that when

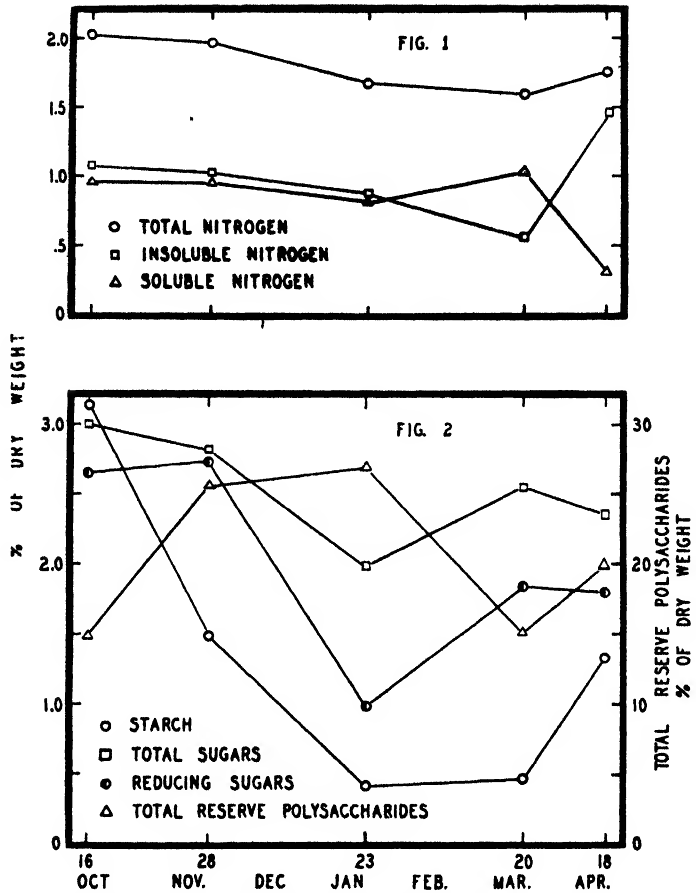

FIG. 1. Changes in total, soluble, and insoluble nitrogen in the buds and one-year-old twigs of yellow poplar during the fall and winter of 1936–37.

FIG. 2. Changes in reducing sugars, total sugars, starch, and total reserve polysaccharides in the buds and one-year-old twigs of yellow poplar during the fall and winter of 1936–37.

sample 5 (April 18) was taken, the buds were just breaking open and some stem elongation had occurred. Some leaves had appeared, and they were about a centimeter in breadth.

During the period from October 16 to January 23 there was a gradual loss in organic nitrogen. This may be a real loss of nitrogen from the twigs and buds, or, more plausibly, merely the dilution of the organic nitrogen present by the accumulation of increasing amounts of reserve polysaccharides. The small gain in the total nitrogen shown for the period from March 20 to April 18 might be attributed to the movement of soluble nitrogenous compounds from the older twigs and branches into the expanding

TABLE I

PERCENTAGE COMPOSITION OF BUDS AND ONE YEAR OLD TWIGS OF YELLOW POPLAR ON A DRY WEIGHT BASIS

FRACTION	DATE				
	OCT. 16	NOV. 28	JAN. 23	MARCH 20	APRIL 18
	%	%	%	%	%
Soluble nitrogen	0.96	0.95	0.81	1.03	0.31
Insoluble nitrogen	1.07	1.02	0.87	0.56	1.46
Reducing sugar	2.65	2.73	0.98	1.84	1.80
Total sugars	2.99	2.81	1.98	2.55	2.36
Starch	3.14	1.49	0.41	0.46	1.34
Total reserve polysaccharides	14.85	25.61	26.89	15.11	19.91

buds (3). During the period from January 23 to March 20 there was a conversion of much of the insoluble nitrogen into more soluble forms. In the period from March 20 to April 18 these soluble compounds were converted into insoluble compounds, probably proteins for the production of new protoplasm in the cells of the rapidly expanding buds.

All of the carbohydrate fractions with the exception of the total reserve polysaccharides seem to be used in respiration. When the sugars and starch were reduced materially in amount, there was a decrease in the amount of total reserve polysaccharides of about 10 grams *per centum,* and at the same time there is an increase in the amount of total sugars of only 0.5 grams *per centum.* Since the trees were dormant at this time, any photosynthetic activity by the chlorophyllous tissue of the buds would not be sufficient to account for any increase in dry weight which might cause such an apparent decrease in the total reserve polysaccharide content. The only tenable hypotheses are that some of these complex carbohydrates or derivatives of them were utilized in the respiratory processes of the twigs and buds, or that they were converted into soluble compounds which were then transported to other portions of the plant. It is also possible, as suggested by O'DWYER (4) that the hemicelluloses are intermediate compounds in the synthesis of lignin from the pectic compounds, and this loss of hemicelluloses might then be attributable to a continuing lignification of the woody tissues. The utilization of the hemicelluloses as a reserve carbohydrate has previously been reported by JONES and BRADLEE (1) in sugar maple, and by MURNEEK (2) in apple spurs. WINKLER and WILLIAMS (5), however, were not able to demonstrate the metabolic utilization of hemicelluloses in shoots of grape vines dying because of repeated defoliation. The latter investigators concluded that hemicellulose probably functions as a structural material and not as a reserve food material in grape shoots. In the period between March 20 and April 18 the small leaves extending beyond the bud

scales were probably active photosynthetically, as is evidenced by the formation of starch and by increase in the amount of hemicelluloses during the period.

Summary

1. Some of the chemical changes occurring in the buds and one-year-old twigs of yellow poplar during the winter, and on breaking of dormancy are reported.

2. There was an apparent decrease in total nitrogen. During February and early March much of the protein was converted into more soluble compounds which were resynthesized into insoluble forms, probably proteins for the production of new protoplasm in the expanding buds.

3. All of the carbohydrates appear to be used in respiration, starch and the sugars most readily, and the hemicelluloses least readily, if at all. With the resumption of photosynthetic activity starch appears, and the hemicelluloses are synthesized again.

The writer is indebted to Dr. PAUL J. KRAMER for many helpful suggestions during the course of this study.

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BRIEF PAPERS

A PRESS FOR RECOVERY OF FLUIDS FROM PLANT TISSUES[1]

T. C. BROYER AND A. H. FURNSTAL

In the course of research upon expressed plant tissue fluids a press was constructed incorporating features of earlier models used in our laboratory. Since this apparatus may be of interest to other investigators, we present a detailed reproduction of the design (fig. 1).

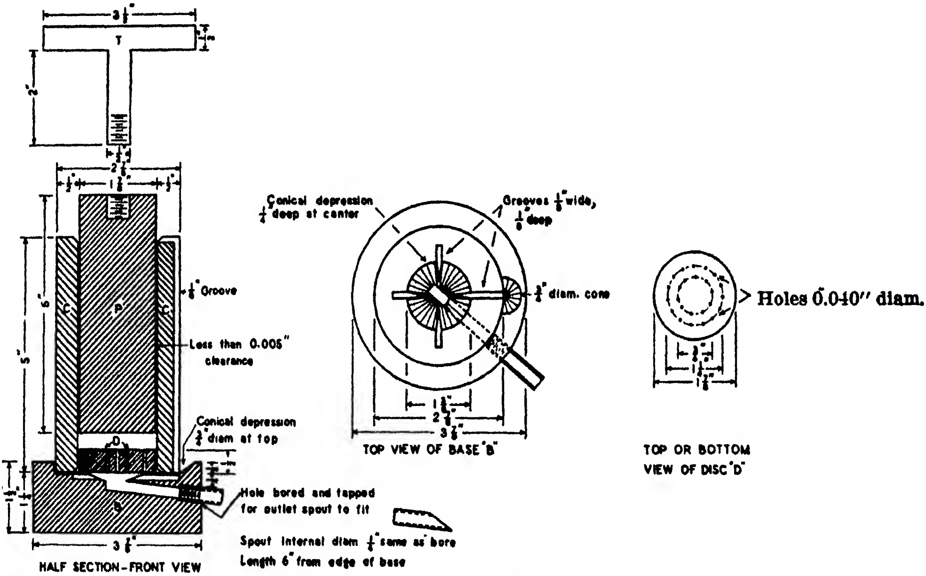

FIG. 1. Details of press for recovery of fluids from plant tissues. B, base; C, cylinder; D, disc sieve plate; P, piston; S, delivery spout; T, T-rod for piston withdrawal.

The dimensions given are suitable for a press to contain about 25 grams of tissue. For larger samples, the diameters of the piston, cylinder, disc sieve plate and base, and thickness of cylinder wall may be increased proportionately. The height of the press should be limited in order to minimize possible internal resistances to compression of the tissues themselves (1). Increase in height may also be limited by the stroke length of the manual screw or working stroke of the hydraulic jack employed for applying pressure.

It is recommended that stainless steel block and tubing be used for all

[1] Clerical assistance in the preparation of this publication was furnished by the personnel of Work Projects Administration Official Project No. 65-1-08-91-B-10.

parts. Materials with chemically resistant characteristics minimize the solution of metals by the expressed fluids. The metals might prove deleterious in subsequent use of these fluids. To avoid electrolytic action between the component parts of the system, dissimilar metals (as steel and brass) should not be used.

The clearance between the plunger and cylinder is of necessity small. The piston may increase in diameter under compression unless made from very hard materials. If the resistance of the steel to penetration is not sufficiently great and if the elastic limit is exceeded certain component parts of the press may become "frozen" to one another. It is essential, therefore, that those parts which are subject to pressure or motion, or those adjacent to moving parts, be fabricated from metals of likewise favorable physical characteristics.[2] In order to reduce the hazard of the plunger becoming frozen to the cylinder, it is obviously necessary that the pressure be applied directly perpendicular to the piston and that the tissue sample be uniformly distributed within the press.

The delivery spout is removable from the screw tap in the base. When fully inserted it should fit flush with the drilling of the base, and the end taper should then be in the upright position. The thickness of the base should be such that the delivery spout clears any object upon which the base is placed.

If the piston can not be manually removed from the assembly it may be withdrawn with the aid of a T-rod inserted in the threaded tap in the top of the piston (**2**). If, however, the pressure is applied from above by means of a screw without an attached platen base, the threaded tap in the piston may be omitted in the construction of the plunger and a hard-metal cupped bearing may be inserted between the screw and the piston during operation.

As shown earlier (**1**, **2**), filters of cheese cloth or muslin enclosing the tissue or inserted as a disc between the sample and the sieve plate aid in the expression of a clear relatively non-colloidal fluid.

In order to recover all of the available liquid expressed, we have avoided the insertion of a sap groove in the cylinder wall and tap hole in the sieve plate. The press assembly is so arranged that it may be conveniently and safely tipped, at the conclusion of pressing, to allow complete drainage of sap.

A heavy wire ram-rod covered with cheese cloth may be employed to dry the delivery spout between samples. The channels in the sieve plate are easily cleaned of compacted tissue residues by reaming out with an ordinary paper clip or needle. All parts are dried with moderate heat

[2] "U. S. S. chromium-nickel alloy steel 18–8" has been suggested as possessing favorable chemical and physical characteristics.

and stored in a closed chamber to prevent corrosion by fumes from the laboratory.

The authors are indebted to several members of the Division of Plant Nutrition for suggestions relative to the construction of this instrument.

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SEED IMPRESSIONS ON PLASTIC FILMS

THORA M. PLITT

(WITH ONE PLATE)

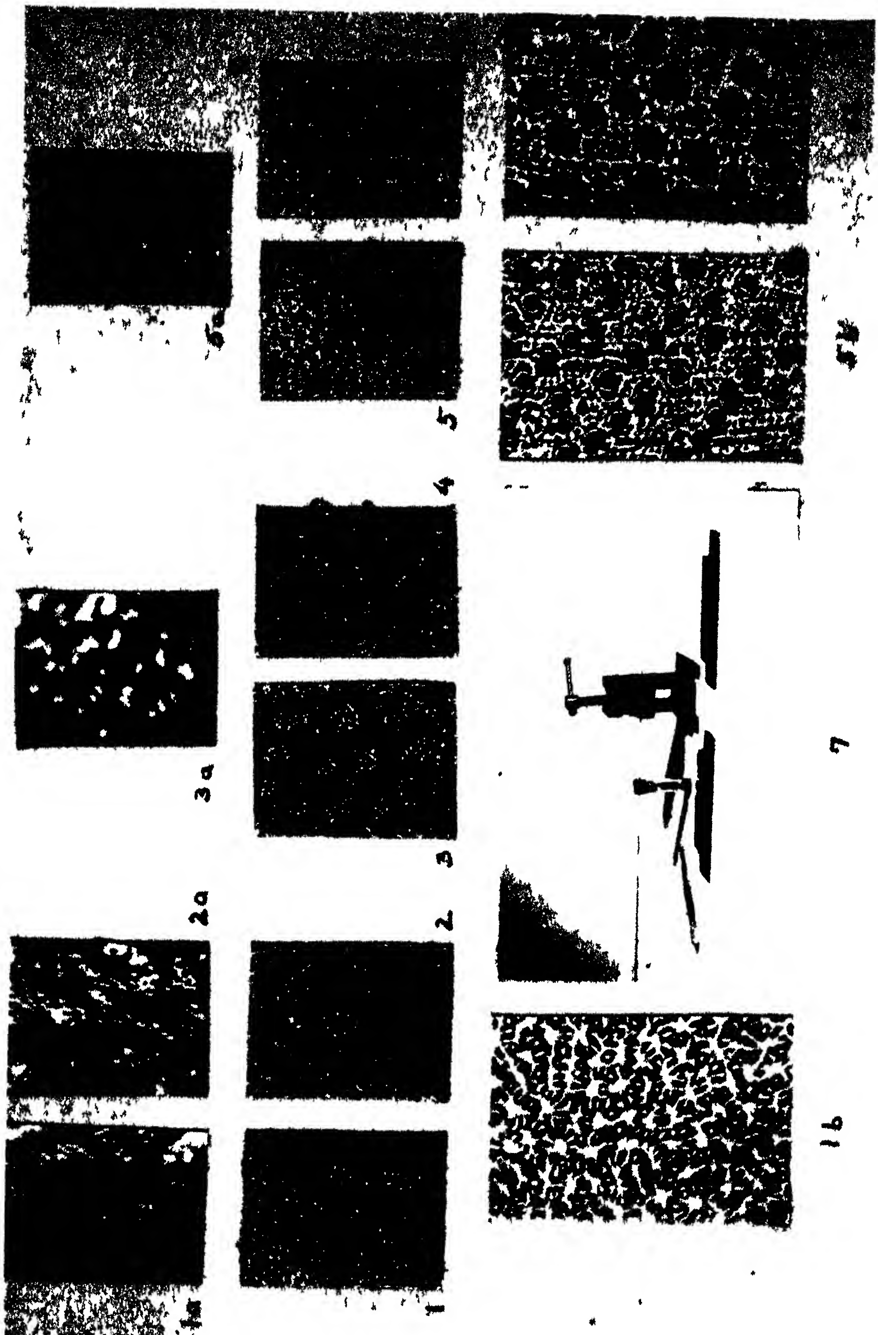
Microscopic details on the hard surfaces of seeds or other parts of fruits are frequently sufficiently characteristic to permit the accurate identification of related species. These taxonomic features have hitherto been largely neglected, however, because of the difficulty of seeing them directly under the microscope. The thickness of the fruit structures, the presence of pigmentation, and the reflection of light from smooth surfaces are difficulties that may be overcome by making impressions of the structures on plastic films. This new method is an outgrowth and modification of a method of obtaining impressions of the surface structures of fur fibers recently developed by HARDY and PLITT (1).

Seeds were placed in carbitol acetate[1] in a small dish for a minute or two. They were then removed by means of forceps and placed between two pieces of transparent cellulose acetate film, 0.005 inch thick and 1 inch square. Adequate padding to obtain clear impressions without crushing the seeds was built up by placing on each side of this double stratum the following: 4 additional strips of plastic film, one strip of sponge rubber (insulation stripping) approximately 0.25 inch thick, and a smooth steel plate. The whole stack was held together by means of a wide clamp which was screwed down until the rubber was compressed to $\frac{2}{3}$ or $\frac{1}{2}$ of its original thickness. This unit was placed for 5 minutes in an electric oven maintained at approximately 90° C. Upon removal from the oven the central double stratum containing the seeds was taken out of the unit and allowed to cool and harden before removing the seeds with a brush or needle. The two plastic films bearing the impressions were mounted face down on glass slides with very narrow strips of transparent adhesive film around the edges. Thus the impressions are positive images of the surfaces. The impressions are best viewed through the microscope using oblique transmitted

[1] Carbitol acetate may be obtained from the Carbide and Carbon Chemical Corporation, 30 E. 42d Street, New York, N. Y.

PLATE V

Photomicrographs of impressions (100 ×) of the achenes of (1) *Polygonum punctatum*, and (2) *P. persicaria;* of the seeds of (3) *Brassica juncea*, and (4) *B. napus;* and of the lemmas of (5) *Poa compressa*, and (6) *P. pratensis*. Photomicrographs of the corresponding parts of the fruits taken directly (100 ×): (1a) *Polygonum punctatum*, (2a) *P. persicaria*, (3a) *Brassica juncea*, (5a) *Poa compressa*. Enlargements of the above impressions (300 ×): (1b) *Polygonum punctatum*, (5b) *Poa compressa*, and (6b) *P. pratensis*. (7) Photograph of equipment used in making impressions.



light, with an amber filter. The 10× objective has considerable depth of focus; magnifications of 100 to 300 diameters are obtained by interchanging suitable oculars.

In this procedure the purpose of placing the seeds in carbitol acetate is merely to use a minimum quantity of the liquid to soften the film. Variations in the procedure for convenience may easily be introduced to suit the material or the equipment at hand, such as the thickness of film, thickness of padding, time, temperature, and manner of controlling the temperature.

The accompanying illustrations show the microscopic details of some seeds or other fruit structures. These materials were furnished by the Section of Food Habits of the Fish and Wildlife Service. The three sets of related species were selected because of their similarities and attendant difficulties of identification, because of their importance in food-habit studies, and in agricultural problems. It may be noted that unsuspected details are revealed by the impressions, and furthermore that the arrangement of characteristic features is visible over a greater area than by direct inspection of the seeds. Clear definition of fine details at higher magnifications may be obtained by use of the impression method. Patterns once established at high magnifications are subsequently more readily recognized at lower magnifications.

This method is applicable in taxonomic studies, in the determination of certain foods and drugs, and in the identification of difficult seeds and fruits in food-habit studies. Impressions may be obtained in a few minutes; furthermore many impressions may be made simultaneously on one set of films.

U. S. Department of the Interior
Washington, D. C.

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COMPARATIVE TRANSMISSION SPECTROGRAMS OF DIFFERENT CONCENTRATIONS OF LEAF EXTRACT

LEWIS W. WEBB, JR. AND FREDERICK F. FERGUSON

(WITH THREE FIGURES)

This study continues the work upon the physical properties of leaf extracts and is a preliminary effort to show the effects of increased dilution of an alcoholic solution of leaf green. FERGUSON, DE LOACH, and WEBB (*loc. cit.*, p. 560, fig. 2) have indicated that dilution of the ethyl alcohol stock solution produces changes in the transmission spectrogram giving an increase in both transmission and absorption regions.

The stock solution used in this study was prepared in the following manner: methyl alcohol (100 ml.) was added to selected fresh green leaves of *Poa pratensis* (3 gm.) and the resulting mixture was heated for 20 min. on a water bath under a reflux condenser. The rather dark green alcohol solution was then decanted. This stock solution considered as 100 per cent. (curve no. 1, fig. 1) was then diluted with methyl alcohol giving these percentages: 50 per cent. (curve no. 2), 25 per cent. (curve no. 3), 10 per cent. (curve no. 4), and 1 per cent. (curve no. 5). The Coleman Regional Spectrophotometer was used to obtain all graphs in the study. The points recorded in each curve represent the average transmission of bands 30 mμ in width.

It may be noted that the form of the curve of transmission of an ethyl alcohol solution of leaf extract is essentially the same as that given by the stock solution (curve no. 1) of this study. There is a tendency to adhere to this characteristic form as shown by the five curves of figure 1. There is relatively little transmission in the region of 350 mμ to 450 mμ in solutions of 100 per cent., 50 per cent., and 25 per cent. Transmission in this area increases sharply in the 10 per cent. solution which displays a marked inclination at *ca.* 430 mμ. Curve no. 5 shows a relatively higher percentage of transmission for the region of *ca.* 350 mμ to 450 mμ than do the other curves. As is expected, the transmission peak at *ca.* 520 mμ increases as the solution is diluted. Curve no. 5 yielded by the 1 per cent. dilution, which was so weak as to be visually indistinguishable from methyl alcohol, still maintains some semblance of this peak. As dilution increases the characteristic "absorption band" as shown by the sharp inclination at *ca.* 660 mμ becomes less accentuated. Curve no. 5 retains but small evidence of this major feature. All curves agree in their sharp inclination at *ca.* 660 mμ and in the leveling off in the infra-red.

The minimum transmission of 0.4 per cent. appears at *ca.* 360 mμ (curve no. 1) while the maximum transmission of 99.6 per cent. appears at *ca.* 740 mμ (curve no. 4). The spectral range extends from 360 mμ to 950 mμ.

In analyzing the comparative graphs shown in figure 1, the peak, at *ca.* 530 mμ, was explained. It was found that the rate at which the transmis-

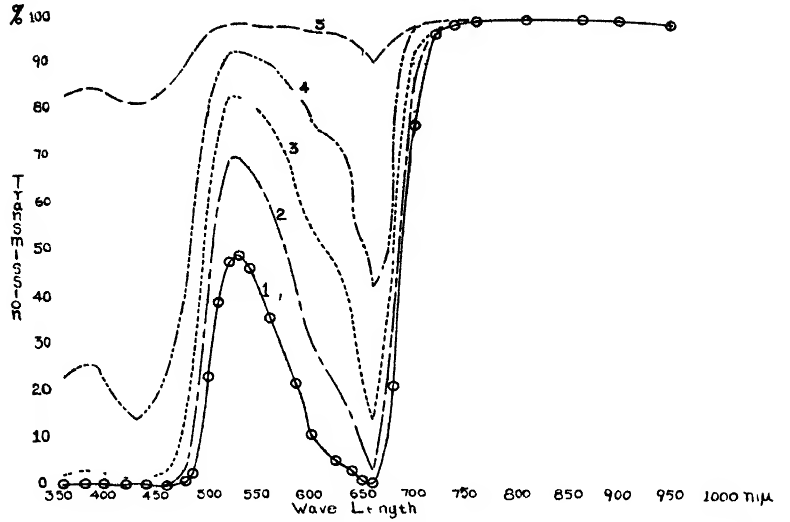

FIG. 1. Comparative graphs showing effects of dilution upon the transmission spectrograms of alcoholic leaf extracts.

sion increases in respect to the decrease in concentration of the solution follows a well defined exponential equation.

Equation no. 1

$$T = 99.65\ e^{-.007\,D}$$

where: T = percentage of transmission
D = percentage of concentration
e = Naperion base

The following figure represents this graphically:

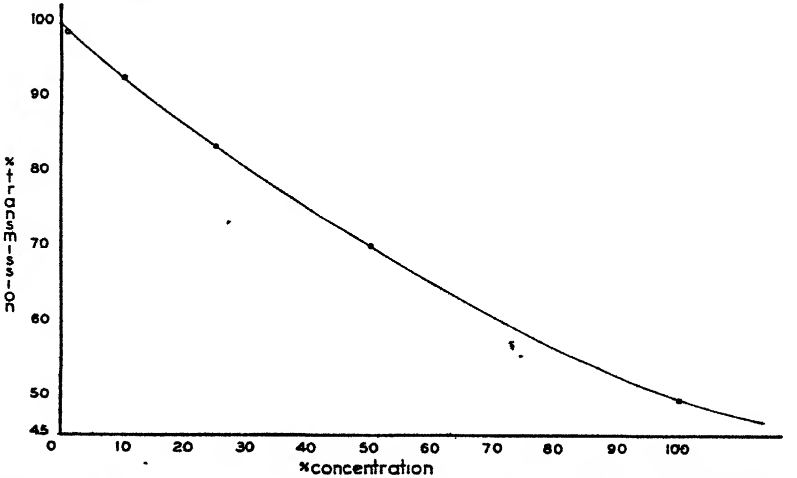

FIG. 2. Graph showing increase in transmission with decrease in concentration of a solution of alcoholic leaf extract for *ca.* 530-mμ wave band.

The trough of the graphs of figure 1, at *ca.* 660 mμ, was also studied. It was ascertained that the rate at which transmission increases in respect to the decrease in concentration follows a hyperbolic equation.

Equation no. 2

$$(d + 8.6)(T + 7.3) = C = 926$$

where: T = percentage of transmission
D = percentage of concentration
C = constant

The following figure shows this relation graphically:

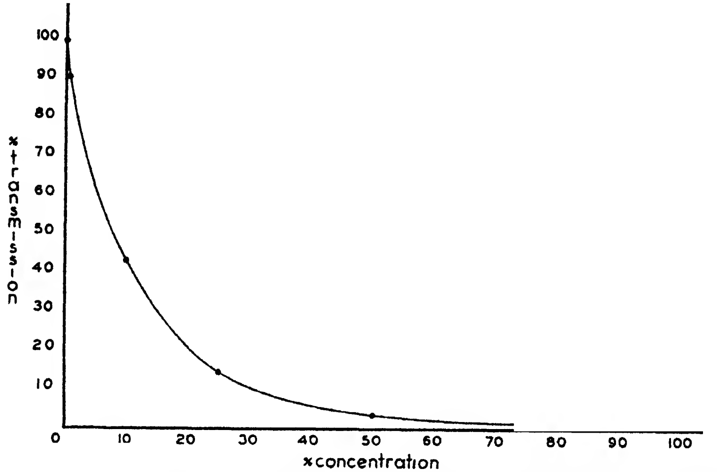

FIG. 3. Graph showing increase in transmission with decrease in concentration of a solution of alcoholic leaf extract for *ca.* 660-mμ wave band.

Further comparative studies on the effect of dilution upon leaf extracts are considered by the authors.

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U.S.P.H.S. CRANEY ISLAND LABORATORY

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NOTES

Southern Section.—The second annual meeting of the Southern Section of the American Society of Plant Physiologists was held at Atlanta, Georgia, on February 5 and 6, 1941, with the Association of Southern Agricultural Workers. The 1942 meeting will be held in Memphis, Tennessee. The attendance was very gratifying, for the increase over the first meeting in 1940 was over 300 per cent. Forty-five were present, there were three sessions instead of one, and 23 papers were read. Membership has more than sextupled during the year, as there are now about 75 members.

These signs of vigor and responsiveness of the southern scientific men to their opportunities are encouraging to the officers who have been responsible for the early steps of this growing organization. The prospects are that the section will grow and aid in a most energetic expansion of research in the South, which is now ready to move forward as the most rapidly developing region in the United States.

A high spot in the program at Atlanta was a round table discussion arranged by Dr. I. E. Miles, of Raleigh, North Carolina, on "The General Aspects of Plant Nutrition." The theoretical aspects were discussed by Dr. J. R. Jackson, Auburn, Alabama; the general application of these problems in agriculture by Dr. L. D. Baver, Raleigh, N. C.; the application to horticulture, by Dr. E. M. Emmert, Lexington, Kentucky; and the commercial aspects, by Mr. Sam F. Thornton, Norfolk, Virginia.

Officers for 1941–1942 were elected as follows: Dr. L. H. Flint, Baton Rouge, Louisiana, Chairman; Mr. Sam F. Thornton, Norfolk, Virginia, Vice-Chairman; Dr. C F. Moreland, Baton Rouge, Louisiana, Secretary-Treasurer.

The section can now look forward to ever-increasing influence in the development of research in a potentially rich agricultural region.

New England Section.—The New England Section has deferred its customary May meeting in order to join with the parent society which holds its summer meeting this year at the University of New Hampshire, Durham, N. H., June 25–26, 1941. This institution is celebrating the seventy-fifth anniversary of its founding, and is anxious to extend its hospitality to plant scientists. June is a pleasant month in New England; college classes will be over; plan to meet your friends in Durham, and relax in the Granite State. Information about the meetings, and accommodations for visitors may be obtained from Dr. T. G. Phillips, the University of New Hampshire, Durham, New Hampshire. It is hoped that there may be a large group of visitors from outside regions who desire to know their New England colleagues better, and to see their fine facilities for research.

Western Section.—The Western Section will hold its annual meeting for 1941 with the Pacific Division of the A. A. A. S. at the California Institute of Technology, Pasadena, California, from June 16 to June 21. Scheduled are symposia on Nutrition (chairman Dr. D. R. Hoagland); Plant Hormones (chairman Dr. F. W. Went); Protoplasm (chairman Dr. O. L. Sponsler). These symposia are invitational programs, and will be held jointly with other societies. An excursion to the Rancho Santa Ana Botanic Garden, and another to the U. S. Regional Salinity Laboratory at Riverside, California, have been planned. Approximately three and one-half days have been set aside for submitted papers. Titles should reach Dr. J. van Overbeek, secretary of the Western Section (California Institute of Technology, Pasadena), not later than the end of April.

Jethro Tull Portrait.—It is a great pleasure to present our readers with the fine portrait of Jethro Tull in this number of Plant Physiology. It was printed fifty years ago by the Royal Agricultural Society of England. It was made from a contemporary painting which at that time, 1891, was in the possession of Mr. Martin J. Sutton. The painting has had an interesting history. It was possibly painted by Allan Ramsay, a contemporary painter who knew of Tull and his work. It was owned a century ago by Mr. John Richards, of Camden House, Reading. In 1840, at the time Mr. Richards died, the portrait was purchased by Mr. John Snare, of Reading; in turn his widow sold the portrait to Mr. Sutton, who has preserved it in the public interest. Speaking of this portrait, Earl Cathcart describes it in the following words: "Fancy him at the age of fifty-six . . . arrayed in his best ruffled velvet coat and full-bottomed wig; and having dined well, he is depicted as demonstrating, with index finger extended and self-satisfied expression, the excellences of some excellent part of his agricultural system —a charming discourse, spiced no doubt with humor, and delivered with the ease of a scholarly gentleman, together with the dignity of an intuitive philosopher." This seems to be a very good interpretation of the portrait, and one can almost hear Tull discussing his hoeing husbandry, with persuasive and authoritative emphasis.

Errata.—Attention has been called to some errors in the issues of 1940, and we are glad to report these to our members, with thanks to those who discovered and reported them. Please note the following:

P. 468, line 2, for "*Elyonurus*," read *Elymus*.

P. 484, citation 9, for "*Elyonurus*," read *Elymus*.

P. 545, citation 3, for 93:, read 63:.

P. 719, line 9 from bottom, for "(8)," read (7).

P. 719, line 2 from bottom, for "(7)", read (6).

P. 720, first paragraph on preparation of reagents, read as follows: Fehling's solu-

tion A was prepared according to MUNSON and WALKER (3); solution B was prepared according to the modification proposed by QUISUMBING and THOMAS (5) as follows:

P. 124, line 6, for "precipitate with," read precipitate. With

P. 724, line 7, for "bottle add," read bottle, break up the precipitate, centrifuge, and decant excess wash water. Add

P. 725, citation 3, for "THOMAS," read WALKER.

P. 771, line 3 from bottom, for "English," read French.

Fellowship for Women.—The announcement of a new fellowship for women, offered by Sigma Delta Epsilon, Graduate Women's Scientific Fraternity, reached us too late to be announced in the January number of PLANT PHYSIOLOGY. As the award would be made before this announcement could reach our members, it is mentioned now mainly to draw the attention of our members to this enlightened and courageous undertaking by Sigma Delta Epsilon. This splendid organization is celebrating its 20th anniversary, and is saying it by thinking of others, perhaps in memory of their own struggles to obtain adequate opportunities for research after the Ph.D. degree had been obtained.

The completion of this project under Dr. LOIS LAMPE, of Ohio State University, National President in 1940, is followed by consummation under the presidency of Dr. DOROTHY DAY, of Smith College, National President in 1941. The stipend mentioned in the announcement is $1000 to $1500. The holder must have the equivalent of the Ph.D. degree, and pursue research either in mathematical, physical, or biological science. She must devote her entire time to the approved research project, or obtain written approval of the Board for any deviation from this obligation.

Sigma Delta Epsilon is the only women's organization affiliated with the A. A. A. S., and is represented by 14 chapters, with about 2500 members. The organization has a right to be proud of its accomplishments, and is setting a challenging example of unselfish devotion to science, and to the welfare of women in research. They deserve hearty congratulations and commendation for their efforts.

Photosynthesis.—It is 15 years since a monograph on photosynthesis has appeared. During this long period much progress has been made, especially in the chemical study of chlorophyll, methods of analysis, and physical-chemical approaches to the problems of carbohydrate synthesis by plants. We are now presented with a monograph by E. C. C. BALY, Emeritus Professor of Chemistry in the University of Liverpool. It bears the simple title *Photosynthesis*, and is published by D. Van Nostrand Co., 250 Fourth Ave., New York. The work records Professor BALY's experiences and deductions from a long series of studies involving concepts of physical chemistry. It deserves, and will no doubt receive, critical examination by

all workers engaged in this field. BALY claims to have brought about a genuine photosynthesis *in vitro,* and there has been some criticism of his methods and deductions while his papers were appearing. In this welcome volume we now have a full and mature statement of his views, based upon his researches over a long period of years. Naturally it raises the whole question of whether one could assume, even if *in vitro* synthesis were accomplished, that it shed any light upon the naturally occurring processes in leaves.

There are eight chapters, with titles as follows: Photosynthesis and the difficulties of its interpretation; the action of ultra-violet light on aqueous solutions of carbon dioxide; the polymerization of formaldehyde into glucose; the photosynthesis of carbohydrates by the action of light on hydrated carbon dioxide adsorbed on a surface; the final achievement of photosynthesis of carbohydrates; the assimilation of nitrogen by the living plant; the mechanism of photosynthesis; and the kinetics of photosynthesis. There are brief author and subject indices.

It is written in very readable style, and should stimulate others to work on these phases of the synthetic processes. It is gratifying to have this summation of Professor BALY's life work, all brought together in a compact statement, where it can be viewed as a whole. Plant physiologists will want to read it carefully and thoughtfully. It is a work of 248 pages, with 24 illustrations, and may be obtained from the publishers at $4.25 per copy.

Enzyme Research.—Lieferung 5 of this monumental work, *Die Methoden der Fermentforschung,* has been received from the press of Georg Thieme, Leipzig. It contains 560 pages, and the "Auslands" price for this number is R.M. 42. Its contents may be briefly summarized, as follows: Active enzyme preparations from algae, yeasts, and molds; from lower animals, particularly the one-celled animals, and from both animal and plant tissues.

The monograph then takes up the isolation and characterization of lyo- and desmo-enzymes; general procedures for concentrating enzymes and for their separation from one another; and the determination of certain general properties of enzymes. These include the splitting of enzymes into their components; direct and indirect proof of the presence of certain atomic groups in enzymes; and the affinities of enzymes for their substrates, reaction products, and other substances.

An appendix to part I, which is concluded here, deals with the effects of radiations on enzymes, and enzyme reactions in heavy water.

The second major division of the work (Zweiter Hauptteil: Spezieller Teil) begins on page 1547. It takes up first the hydrolases, which include the following: Lipases, cholinesterase, tannase, chlorophyllase, phosphatases

(both plant and animal), phytase, lecithase, and sulphatases. The stereochemical specificity of ester-splitting enzymes, and the synthesizing effects of ester-splitting enzymes come in for treatment at the close of the hydrolases of the esterase type.

The last portion of Lieferung 5 takes up the carbohydrases. There is a general treatment of these, then saccharase and invertin, maltase, b-glucosidase, galactosidases, trehalase, digilanidase and other specific glucosidases, heteroglucosidases, glucuronidase, and thioglucosidases (myrosin group) complete the 5th Lieferung.

This brings the total work to page 1836, about half of it, or possibly more than half. BAMANN and MYRBÄCK are making a great contribution to science in providing such a source book of information. Enzyme workers of the world will owe them a debt of gratitude for bringing out this excellent compendium on enzyme research.

Mineral Deficiencies.—Plants are now known to exhibit symptoms of mineral deficiencies in widely scattered areas throughout the United States. While the symptoms are more frequently observed in regions of loose soils and heavy rainfall such as the coastal plains, almost any region may show deficiencies of elements needed in mere traces. It has become necessary for the physiologist to recognize these symptoms at sight, and to be on the lookout for them everywhere. The prompt recognition of specific responses to specific deficiencies is important, and any helps toward this end are important. Under the title: *If They Could Speak*, the Chilean Nitrate Educational Bureau, Mr. HERBERT C. BREWER, Director, 120 Broadway, New York, has issued a booklet with many color reproductions of plant mineral deficiency symptoms. They were made from Kodachrome transparencies, and truly, the plants speak for themselves. There are 95 reproductions, two per page with one on the front cover, and they carry brief explanatory legends. The deficiencies illustrated concern magnesium, boron, manganese, copper, zinc, iron, potash, calcium, and nitrogen. About 35 crops are included in the survey, each showing one or more deficiency diseases. Some of the more important crops are cotton, corn, oats, tobacco, apples, citrus fruits, soy beans, tomatoes, cabbage, cauliflower, celery, etc.

This excellent educational booklet, 54 pages, can be obtained free on request from the director of the Bureau. It should be in the hands of every plant physiologist. Mr. BREWER would also like to obtain additional portraits that would extend the range of crops or minerals, and any suggestions from those with first hand experience with deficiencies will be appreciated.

General Botany.—We have received from Barnes and Noble Inc., 5th Ave. and 18th St., New York, a copy of *An Outline of General Botany* by

Harry J. Fuller, of the University of Illinois. This is one of the College Outline Series, published by Barnes and Noble, contains 180 pages, 49 text figures, and an index. It retails for 75 cents, with discounts on class supply orders. It offers a rapid survey of the main facts of general botany, and the generalizations which have been achieved in this field. It is a very helpful aid for private study.

WINTHROP JOHN VANLEUVEN OSTERHOUT
AUGUST 2, 1871

THIS NUMBER OF PLANT PHYSIOLOGY

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SIGNIFICANT RÔLES OF TRACE ELEMENTS IN THE NUTRITION OF PLANTS[1, 2]

JOHN W. SHIVE

During the past quarter of a century much has been written concerning the place of the so-called trace elements in the economy of plant life. Most of this literature deals with the effects produced upon plants when these elements are absent from the growth substrate and is purely descriptive in nature, portraying the symptoms of deficiency. There is also, in the physiological literature of the past decade and a half, much descriptive material portraying the pathological symptoms which appear in plants when these elements occur in the plant environment in concentrations in excess of those required for growth and development. Thus far these qualitative observations make up practically all of the great mass of the investigational literature upon this subject. So much of this foundation in natural fact has already been accomplished, that intensive activity along this line of categorical description can no longer be considered an adequate field of investigation in this particular phase of plant nutrition. It should be remembered, however, that the extension of our knowledge in this phase of nutrition cannot progress without at least some continued activity along these descriptive lines; but our chief interest is no longer in the qualitative pathological symptoms themselves, which develop as the result of trace element malnutrition, but in their relation to those more basic phenomena which can be evaluated only through careful quantitative experimental investigation. From almost the beginning of the present century studies have been directed toward ascertaining whether or not certain elements, which are found in trace quantities in both the plant and in its environment, might be included in the category of essential factors for growth and development. There is much contradictory evidence, as might be expected, relative to this question, which

[1] Journal series paper of the New Jersey Agricultural Experiment Station, Department of Plant Physiology.

[2] The sixth STEPHEN HALES address, read before the American Society of Plant Physiologists at Philadelphia, December 30, 1940.

is an important one. The energetic search for such elements which began early in the century is still in progress. It has been in the past a profitable field of investigation and it is predictable that it will provide an equally profitable field of study in the future. The inability of experimenters to grow plants successfully through a co[illegible]cle in artificial culture, in the early years of the present century, in[illegible] a search for missing essential factors. The usual experimental technique which had long been employed in solution culture and in sand culture was too crude, however, and the materials used were too unrefined to disclose the need of specific elements required in minute quantities in nutrient substrates for plants.

Search for the missing factors, therefore, led to great improvements in experimental techniques. Great strides have been made in the refinement of methods and in the purification of nutritive materials for accurate study in the endeavor to demonstrate the need of these elements in the nutrient substrate and to prove their essentiality. Through this refinement of technique, improvement in the methods of operation, and purification of nutritive material, it is now possible to add to the previous list of essential elements for plants boron, manganese, zinc, and copper. Undoubtedly other elements will be added to this list with still further purification of chemical materials and further refinements of physical equipment and methods of procedure. The degree of refinement at present attainable, however, imposes a rigid limitation upon experimental accuracy and this is particularly true with respect to the analytical data from quantitative studies in the field of trace element nutrition. The suggestion has recently been made that an element may not be regarded as non-essential if it is present in a nutrient substrate in concentrations greater than one part in a billion (**10**). Such a degree of purity may not be attainable at the present time. It is this point, as well as that relating to the absolute importance of the trace elements in nutrient substrates, which has recently been so strongly emphasized in connection with the development and refinement of methods for growing isolated plant tissues *in vitro* (**12, 13**). It appears that the degree of purity and the state of refinement are determining factors in any attempt to establish proof of the essentiality of a given element which is present in the plant environment in very minute concentration. It is not so simple a matter to differentiate between that which merely stimulates growth and that which is essential for growth. The fact that the presence of a minute trace of a given element in a nutrient substrate may stimulate growth of the plant does not constitute proof of essentiality, nor does failure to stimulate constitute proof that an element is not essential. In order to provide proof of the essentiality of a given element it is necessary to show that a plant cannot complete its cycle in the absence of a measurable concentration of the element in question.

In order to obtain quantitative information concerning the place which the trace elements occupy in the nutrition of plants and the rôles which they play in metabolic processes, elaborate precautions must be taken to eliminate all possible sources of contamination derived from the experimental set-up, and careful attention must be given to such matters as the purity of salts, distilled water, and cleanliness of the apparatus involved. These precautions are indispensable if the objectives of quantitative research in this field are to be attained. With the discoveries made during recent years, and with the refinement of the experimental method, it has now become a simple procedure to demonstrate the effect of deficiencies of boron, manganese, and zinc with many species of plants under the conditions of control which characterize the usual routine of solution culture experimentation.

While superficial descriptions relating to the effects of trace element deficiencies upon the various species continue to accumulate, as is to be expected, it is most encouraging and even inspiring, that a pronounced change toward the more quantitative type of investigation has been in progress during the past several years and some very important quantitative work dealing with trace element nutrition has appeared in the literature (**2, 5, 7, 8**). Difficult as it has been to demonstrate beyond a doubt that boron, manganese, and other trace elements are essential for plants, it may, however, be infinitely more difficult to prove why they are essential. But the present status of trace element nutrition in plants makes it unnecessary to dwell upon either the economic or the theoretical importance of the elements required by plants in only very minute amounts. The economic importance of these elements is obvious in view of the host of plant diseases, many of which are very destructive, which have been traced either directly or indirectly to deficiency or excess of one or more of these elements in the soils of many agricultural districts. It is to be emphasized also that not one of the many problems relating to the important rôles which these elements play in the metabolic processes of plants, has yet completely emerged from the theoretical category to take its place in the realm of fact established through experimental study. The importance of theoretical consideration of such problems is, therefore, apparent.

It has early been pointed out by several investigators that the pathological symptoms produced in plants by boron deficiency are remarkably similar to those produced by calcium deficiency under like experimental conditions. The similarity has been recognized in virtually all of the species thus far studied. This has suggested the possibility that boron is in some mysterious manner involved in those processes in the plant in which calcium is a direct reactant. This suggestion is supported by the well-known fact that both boron and calcium are especially effective in the intensive metabolic processes which are confined largely to the meristematic regions, and recent investiga-

tions have shown also an intensive utilization of boron by the reproductive organs of the plant (6).

Attention will now be directed to the consideration of quantitative experimental evidence relating to an important rôle which boron plays in the accumulation, assimilation, and metabolism of calcium in several species widely separated in their morphological, physiological, and taxonomic characteristics. But, before presenting this evidence it will be necessary to describe very briefly, the experimental procedure by which these data were obtained. Relatively large numbers of plants of each species dealt with were grown in a standard culture medium until they had attained the age and phase of growth and development desired before the experimental treatments were begun. At the beginning of the treatment period the cultures were divided into three groups or series: The cultures of the first series covered a range of boron concentrations in the standard nutrient substrate which, by previous tests, was known to produce visible symptoms of deficiency in the species in question. The cultures of the second series covered a range of approximately optimum boron concentrations; and the cultures of the third series covered a range of boron concentrations sufficiently high to produce symptoms of toxicity in the plants of the species in question. With the exception of the boron treatments the nutrient substrate used in the cultures of the three series was identical. For the sake of convenience the three series may be designated the deficiency series, the optimum series, and the toxicity series, respectively. In all cases the experimental period, during which the cultures were under treatment, was continued until pronounced visible symptoms of boron deficiency appeared in the plants of the deficiency series, and of boron toxicity in the plants of the toxicity series. This required different intervals of time for the different species. The cultures were conducted under a system of continuous flow of the culture solution at a rate sufficiently high to prevent material alteration in the proportions of nutrient ions through the activity of the plants.

At the end of the experimental interval the various tissues of the plants were analyzed for calcium and for boron and an attempt was made to differentiate between total and soluble or active quantities of the elements in the tissues tested, in the hope that such a procedure might throw some light upon the connection between these two elements in the metabolic activity of the plants. While the experimental evidence reveals absolutely no information concerning the actual physiological function of either boron or calcium in the plant, it does indicate at least one important rôle which the trace element boron plays in the functional processes of the major element calcium.

From a consideration of the experimental data (7) it became immediately obvious that the boron in the tissues of the particular species in question (corn) was virtually in a completely soluble state and could therefore be

assumed to be in a mobile, active condition. It was obvious also that its accumulation in the tissues was determined by its concentration in the nutrient substrate. When now the experimental data relating to boron were examined in connection with corresponding data representing the status of calcium in the plant, the outstanding feature which was at once apparent was the almost perfect correlation between the boron of the tissues and the soluble or active calcium of the tissues. On the other hand, there was no apparent relation between the boron content and total calcium in the tissues of this monocot, and there was no obvious relation between soluble and total calcium in tissues of these plants. It must be concluded, therefore, that the proportional part of the total calcium in the plant which was maintained in the soluble, active state in which it could be translocated from points of supply to centers of metabolic activity, was determined not by the total calcium content of the plant but by the supply of available boron in the corresponding tissues which in turn was determined by the boron concentration of the nutrient substrate. This peculiar property of boron by which calcium is rendered mobile and active is not confined to organic substrates. The principle involved is effectively utilized in certain industrial procedures to eliminate calcium in the soluble form from complex inorganic (**3**), as well as organic (**1, 4**) systems in which it occurs as an impurity during the process of large-scale preparation and manufacture of certain important commercial products.

The corresponding analytical data of a representative dicot, *Vicia faba,* were next considered. When these were treated in a manner similar to those of the monocot, corn, it was found that a relatively small fraction of the total boron in this dicot was soluble and in this respect the dicot was strikingly different from the monocot. But the soluble boron fraction of the dicot, like that of the monocot, was directly related to the total boron content of the tissues and also to the concentrations of this element in the nutrient substrate.

In the dicot, *Vicia faba,* the soluble calcium, as in the monocot, was directly related to and determined by the soluble boron fraction, which in turn was related to the total boron content and determined by the concentration of this element in the nutrient substrate. There was no direct relation between soluble and total calcium either in the monocot or in the dicot, but in the toxic range of boron concentrations of the substrate the accumulation of calcium was impeded, and this was directly associated with a corresponding retardation of the actual rates of calcium absorption and of the rates of growth.

One of the characteristic differences between the monocot and the dicot relative to this particular phase of inorganic nutrition was the fact that under identical experimental conditions with respect to the chemical and

physical properties of the nutrient substrate, the total calcium content and the total boron content were always much higher in the dicot than they were in the monocot. At present there is no adequate explanation for this fairly general phenomenon. In all of the species so far investigated the calcium and boron values for the dicot are usually much higher than those for the monocot. But this did not hold for the soluble fractions of these elements in the tissues of the plants. The soluble fraction of boron relative to the total was always much lower in the dicot than in the monocot and this invariably resulted in a low fraction of soluble calcium, relative to the total.

TABLE I

AVERAGE SOLUBLE BORON AND AVERAGE SOLUBLE CALCIUM, PERCENTAGE OF TOTAL, PRESENT IN THE TISSUES OF MONOCOTS AND DICOTS

RANGE OF SUPPLY	MONOCOTS		DICOTS	
	SOLUBLE BORON	SOLUBLE CALCIUM	SOLUBLE BORON	SOLUBLE CALCIUM
	%	%	%	%
Deficiency boron range	62.7	28.3	6.5	16.5
Optimum boron range	78.4	34.7	28.7	22.9
Toxicity boron range	86.1	55.7	32.2	25.4

This is clearly brought out in table I showing percentages dealing with relative solubilities of the two elements in representative species of the two groups of plants, monocots and dicots, so important in general agriculture. A consideration of these data provides an explanation for the well-known fact that the boron requirement of the monocot for normal growth and development is very much lower than is that of the dicot but equally essential for both, and it accounts also for the frequent appearance in the literature of statements to the effect that boron is not essential for growth and development of the agricultural monocots such as oats, wheat, and some grasses: The optimum boron requirements of the dicots thus far investigated were from five to ten times as high as those of the monocot, but this is only a rough estimate, since it is extremely difficult to evaluate accurately, in terms of growth criteria, the effective concentrations of boron in the extremely low ranges required by the monocots.

In the course of these investigations it was shown frequently that if at any stage in the cycle of these experimental plants the boron was excluded from the nutrient substrate so that a deficiency of active boron occurred within the plant, such deficiency rapidly destroyed the potential metabolic possibilities of the calcium even when calcium was present in adequate concentrations both in the tissues and in the substrate. Under such conditions

the plant quickly manifested deficiency symptoms. Were they boron deficiency symptoms or were they calcium deficiency symptoms? Who knows, since a deficiency of available boron in the tissues was directly associated with an inadequate supply of active calcium, and since the symptoms produced by an apparent deficiency of one element were identical with those produced by a deficiency of the other? If, on the other hand, boron was maintained in adequate concentration and calcium was excluded from the nutrient substrate at any stage in the growth cycle, calcium deficiency symptoms did not manifest themselves until the calcium previously acquired by the plant became inadequate in quantity to maintain the normal growth status of the plant. This required a relatively long period of time, since in the presence of an adequate supply of active boron the calcium already in the plant functioned quite effectively.

There is considerable experimental evidence to indicate that boron is a vital factor in the processes involved in organic syntheses. During the course of microchemical investigations of the terminal meristematic tissues of both monocots and dicots, it was discovered incidentally through the use of methods of staining, that striking differences occurred in both the pectin content and the fat content of the cells of the tissues from plants grown in the deficient, the optimum, and the toxic boron concentration ranges. An attempt was then made to determine qualitatively and roughly by quantitative methods whether the differences observed might in any way be related to the effective boron concentrations in the nutrient substrate and in the tissues. It is interesting and perhaps important that plants grown within the range of deficient boron concentration yielded strong positive tests for pectins and negative tests for fats. Plants grown within the range of toxic boron concentrations always yielded negative tests for pectins and strong positive tests for fats. Plants grown in the range of optimum boron concentrations always showed the presence of both pectins and fats. It must be strongly emphasized that these observations and tests are merely suggestive and will require confirmation by repeated checks and tests making use of much more exact methods of quantitative analyses than those employed in these preliminary investigations which strongly suggest that boron plays an important indirect rôle in carbohydrate synthesis and fat metabolism. In such a rôle its effects can not be direct but its influence is made effective through other factors in some such manner as has already been discussed, and these factors may be inorganic in nature, such as calcium involved in the mechanisms of organic synthesis. This becomes understandable in view of the fact that in green plants with high concentrations of active calcium, carbohydrate synthesis may give way to the formation of fats and fatty substances (9). Further experimental evidence also suggests that boron may play similar indirect rôles in certain metabolic processes in-

volving the cations and perhaps the anions of major elements other than calcium (**11**), but this evidence is too fragmentary and inadequate for broad generalization.

There is another important phase of trace element nutrition which involves the activity of the two elements iron and manganese. That these two elements are intimately interdependent in their effects upon the plant, and that the nature of the activities of one of these is determined by the proportionate presence of the other, can no longer be doubted. Such interrelationships between these two elements, relative to their active influence in the metabolic system of the plant, have been suggested by many investigators. These suggestions have usually been associated with the fact that symptoms of chlorosis, and other characteristic effects resulting from deficiency or excess of one of these elements, are either identical with or reciprocally related to each other.

The theoretical explanation of the rôle which manganese plays in the metabolic processes, in which iron also assumes an important rôle, revolves around two facts: first, that the active, functional iron in the tissues is in the reduced state, that is, in the ferrous condition; and, second, that the oxidizing potential of manganese is higher than that of iron. If iron in the ferric state is absorbed by the plant, much of it is immediately reduced to the ferrous form under the powerful reducing systems in the cell, unless it is restrained by some counterreactant. If such a reactant is not present, or is present only in deficient quantity, a very low concentration of iron in the active state may become a powerful toxic agent resulting in a type of chlorosis which is readily recognizable as an iron toxicity symptom, or it might with equal correctness be designated as a manganese deficiency symptom. If, however, a strong oxidizing agent with an oxidizing potential considerably higher than that of iron is present in adequate concentration, the reduction of iron is restrained, or if iron is already present in the reduced state, it may be oxidized to the ferric state, in which condition it may be precipitated, probably in the form of ferric organic complexes. Manganese is the element which possesses the chemical characteristics necessary for such a theoretical system, and there is little doubt that it reacts with iron in the plant in accordance with the chemical principles suggested and in some such manner as is here described, but the sequence of events and the details of the exact processes involved are utterly complex and not at all understood.

Assuming that such a dynamic relationship exists in the plant between iron and manganese, it then becomes evident that so long as a supply of iron and manganese is available in the nutrient substrate a dynamic equilibrium system involving both oxidized and reduced ions of both iron and manganese must be maintained. Theoretically, however, complete oxidation or complete reduction cannot be attained in such a system so long as a supply of

these elements is available in the nutrient substrate and absorbed by the plant; but the oxidation of ferrous ions to ferric ions and accordingly the precipitation of iron in the form of ferric organic complexes is determined by the relative quantity of manganic ions present in the system. Thus, when the relative proportion of manganic ions in the plant is high, the active ferrous ions are maintained at a proportionately low level through this process of oxidation reduction. But when the relative proportion of manganic ions is low, the active iron, which is capable of functioning in the metabolic processes, is maintained at a proportionately high level. When these active ferrous ions become excessive in the tissues through a shift in the dynamic equilibrium system in their direction because of a proportionately low supply of manganese in the nutrient substrate, the plant will manifest this excessive iron activity by the development of toxicity symptoms. On the other hand, when the equilibrium is shifted in the opposite direction, as it is with relatively high manganese in the substrate, so that a deficiency of active iron results, the plant will manifest this by the development of symptoms associated with iron deficiency. These symptoms might with equal correctness be designated as manganese toxicity symptoms.

These processes have been followed analytically both with plant materials and in inorganic systems and the several steps described have been experimentally verified. Their effects upon the plant can readily be demonstrated and this has been done in the following manner: Three series of cultures were grown in a standard culture solution. In each series of cultures the iron supply was maintained at a constant level. This was very low in one series, approximately optimal in the second, and high in the third. In each series the manganese concentration range extended from very low to very high concentrations so that in one direction the only variable factor was that of iron concentration and in the other direction the only variable factor was that of manganese concentration.

Inspection of the analytical data from these series of cultures brings out the fact that a shift from low to high iron in the substrate causes a corresponding shift in the concentration of manganese required to produce good growth of the plants, free from symptoms of toxicity or deficiency. In other words, when iron is low in the substrate good growth is obtained only when manganese is correspondingly low, and when iron is high in the substrate good growth is obtained only when manganese is correspondingly high. The data further show that good growth can be obtained with a relatively wide range of iron concentrations in the nutrient substrate but only when accompanied by a corresponding range in the concentrations of manganese. This demonstrates the importance of maintaining in the nutrient substrate the proper ratio of iron to manganese, which has a value of approximately two (2) for the species investigated although good growth may be obtained

within a limited range of values for this ratio. It demonstrates also, that, within certain limits not attained in these investigations, the total supply of these two elements is not at all important, provided only that the proper ratio of active concentrations of iron to manganese is maintained. The analytical data show that within the tissues of the plant a similar relationship exists, not between total quantities of iron and total quantities of manganese, but between active iron and active manganese, although here a somewhat greater fluctuation than that which is permissible in the nutrient substrate, may occur in the value of this ratio and still be consistent with good growth and development of plants which are free from pathological symptoms. The effective internal range of these ratio values extends from approximately 1.5 to 2.5 for the species investigated, but it is not expected that the same range of values would be effective with all species with reference to either the nutrient substrate or the active plant tissues.

As a final consideration and as the result of these studies, it may be pointed out that the rôles which have here been ascribed to the trace elements boron, iron, and manganese, important as they may be, are mere incidents in the whole complex maze of physiological functions which they may indirectly assume in the vital activities of any species. While it is at present impossible to assign any one particular process as the special function of a given trace element, it is probably safe to assume that each of these elements is a critical factor in every important physiological process involved in the nutrition of a plant.

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WATER BALANCE IN THE ONION ROOT: RELATION OF VOLUME INTAKE TO VOLUME EXUDATE OF EXCISED ROOTS AND ISOLATED ROOT SEGMENTS

HILDA F. ROSENE

(WITH TWO FIGURES)

Introduction

In order to effectively study water balance and ion exchange in higher plants it is important to obtain quantitative data concerning the mechanism of transport in root tissue *per se*. Such knowledge is also of significance in cell dynamics since water balance is a property common to all cells. Although a careful study involving simultaneous determinaton of both absorption and exudation in a single isolated root or portion of root is essential to a complete analysis of the dynamics of polar transport in higher plants, no such study has appeared heretofore. The present investigation which was confined to excised onion roots and isolated root segments should be extended to include other types of roots including those which have been grown by tissue culture technique.

Young onion roots (less than two weeks old) grown in water culture show polar apical-basal differences in rates of water intake which are characteristic of root tissue since these differences are maintained both before and after the roots have been isolated from the bulb and developing leaves; although water intake occurs at all levels, higher rates appear in the relatively more basal regions (**16**).

Unit volume of transport at a given axial level in an excised onion root may be just as large or larger than when it had the supposedly added force of the bulb and developed leaves to reinforce or supplant its own force in the transport of water in a saturated atmosphere. There is, of course, no conclusive evidence which shows whether or not the bulb and developing leaves exert a force which supplants that of the root under the given conditions. Although in some roots the rates are lower immediately after severing the root from the shoot, fifteen hours later the rate of transport by the excised root is frequently greater than that during a previous corresponding intact period. It has been shown (**16**), however, that rates at given axial levels of young roots increase with time in both the intact and excised state.

Fluctuations in the characteristic gradients of water intake occur along the longitudinal axis of intact and excised onion roots. Spontaneous variations in rates, sometimes opposite in direction, are also simultaneously exhibited by contiguous levels from one two-hour interval to another (**15, 16**).

Water loss from epidermal cells has never been observed at any axial level in the healthy growing onion root; but previous experiments have not shown whether or not a decrease in rate was produced by liquid loss from minute cellular areas. The area at a single potometer contact in experiments on several hundred roots was seldom less than 2, nor greater than 4, square millimeters; if liquid loss occurred from minute cellular areas decreasing the total intake at a single potometer contact, such loss was obscured by an algebraic over-all intake at a given level during the interval. JENNY, OVERSTREET, and AYERS (**9**) have demonstrated that intake of ions by some roots is not a unidirectional process and that the same ion species may move into and out of the root at the same time. Other ion exchange studies have also been made; MAZIA (**13**) showed that ion exchange occurs in Elodea; MULLINS and BROOKS (**14**) and BROOKS (**4**) have demonstrated radioactive ion exchanges in single cells. Is water absorption a unidirectional process? Does water loss occur when rates of intake decrease and exudation pressure falls? Does water loss enter into the maintenance of the "rhizosphere" around roots? SIERP and BREWIG (**18**) and BREWIG (**2**) have described water loss in the apical region of *Vicia faba* simultaneous with absorption in basal regions.

The appearance of small irregular oscillations in the "bleeding" of cut stems with root systems attached was recorded by BARANETZKY in 1877 (**1**). In a recent article by HEYL (**7**) similar phenomena are mentioned. No measurements were made by either investigator to determine the possibilities of parallel variations in water intake and exudation. GROSSENBACHER (**5**) has described diurnal fluctuations in root pressure and volume exudation. Measurements of water intake to determine the presence or absence of parallel recurring maxima and minima in both absorption and exudation were not included in his investigations. In studies on the influence of the shoot on root permeability and resistance to water intake BREWIG used root systems with a portion of the stem attached (**2**). In a more recent article BREWIG (**3**) describes experiments with isolated root segments, but the object of the experiments differed greatly from those which are presented in this paper; BREWIG determined the effect of passing air over a portion of the root segment to simulate transpiration; he also used osmotically active solutions. In the present investigation no attempt was made to determine the effect of different agents on water transport. It appears to be the first study to include simultaneous measurements of absorption in contiguous regions and exudation in root tissue *per se* in a saturated atmosphere under constant external conditions. The technique used in the following experiments makes possible the simultaneous measurement of intake, outgo, and retention of water and therefore fulfills the fundamental experimental requirement in an adequate quantitative study of water balance.

Methods and results

WATER TRANSPORT IN EXCISED ROOTS: RELATION OF VOLUME ABSORPTION TO VOLUME EXUDATION

Calibrated potometers of small bore attached in a horizontal position to an upright glass rod within glass chambers provided a means of determining absorption rates and exudation in different root regions at the same time. Experiments were carried out in the dark with a saturated atmosphere inside the chambers. Room temperature did not vary more than $\pm$ 0.5° C. in the longest experiments. Condensed liquid which collected on the observation window was removed before each reading by careful manipulation of a "window wiper" from the outside. Further details of the apparatus may be obtained from previous publications (**15, 16**). Roots were obtained from onions (*Allium cepa*) grown in aerated nutrient solutions or in soil.

Simultaneous measurements of water intake and of exudation of over 100 individual excised roots reveal that fluctuations in both volume of inflow and outflow occur from one two-hour interval to another and that the variations in volume outgo may occur independently of variations in intake. The ratio of volume output (exudation) to volume input (absorption) during any two-hour interval may be equal to, slightly greater, or less, than 1. Characteristic results are represented by the data in table I; additional data would be repetitious.

The plants from which the data in table I were obtained were placed in the experimental chambers the night preceding the experiment; excisions were made the following morning without removing the chamber covers in order to eliminate any marked change in humidity within the chambers. Only a portion of the potential absorbing surface of each root was utilized since not more than four potometers were used for absorption; the absorbing area at a single potometer contact was not greater than 3.69 sq. mm. and not less than 1.45 sq. mm. The potometers were spaced 10 mm. apart with the bottom potometer at the apex. Readings were made at 2-hour intervals throughout a 24-hour period following an initial reading at 10 A.M. Elongation at decreased rates continued in the excised roots from 6 to 12 hours.

All four roots manifested marked oscillations of the ratio of volume inflow (absorption) to volume outflow (exudation) from interval to interval. Independent fluctuations in both input and output were apparent; frequently diminution of intake simultaneous with acceleration of outflow, or *vice versa*, was displayed.

The greatest range of variation of ratios appears in root II, which exhibited a ratio of 0.05 during the second and 1.05 during the tenth interval. All four roots manifested a ratio less than unity during the first interval. This is usually the case immediately following excision. Although precautions were taken to supply the plant with an abundance of water preceding

TABLE I

VOLUME OF TAP WATER ABSORBED FROM FOUR POTOMETERS AND SIMULTANEOUS EXUDATION OF FOUR SINGLE ROOTS DURING TWO-HOUR INTERVALS*

OT	INTERVAL	FIRST	SECOND	THIRD	FOURTH	FIFTH	SIXTH	SEVENTH	EIGHTH	NINTH	TENTH	ELEVENTH	TWELFTH
	TIME	10 A.M. TO 12 P.M.	12 TO 2 P.M.	2 TO 4 P.M.	4 TO 6 P.M.	6 TO 8 P.M.	8 TO 10 P.M.	10 TO 12 P.M.	12 TO 2 A.M.	2 TO 4 A.M.	4 TO 6 A.M.	6 TO 8 A.M.	8 TO 10 A.M.
I	Absorption (mm.^{3})	1.330	1.055	1.119	1.638	1.557	2.139	2.173	2.432	2.273	2.276	2.027	2.171
	Exudation (mm.^{3})	0.389	0.389	0.649	0.649	1.525	1.927	2.109	2.011	2.463	2.312	2.028	2.019
	Ratio $\frac{\text{Vol. Exud.}}{\text{Vol. Abs.}}$	0.29	0.37	0.58	0.39	0.98	0.90	0.97	0.83	1.08	1.01	1.00	0.93
II	Absorption (mm.^{3})	0.733	1.207	0.604	1.173	1.386	1.974	1.828	1.907	2.429	2.368	2.333	2.363
	Exudation (mm.^{3})	0.098	0.065	0.293	0.587	0.978	2.043	1.900	1.830	2.210	2.501	2.340	2.103
	Ratio $\frac{\text{Vol. Exud.}}{\text{Vol. Abs.}}$	0.13	0.05	0.48	0.50	0.71	1.03	1.03	0.96	0.91	1.05	1.00	0.89
II	Absorption (mm.^{3})	1.315	1.200	1.378	1.381	1.135	0.746	0.779	0.617	0.975	0.986	0.908	0.828
	Exudation (mm.^{3})	1.233	1.200	1.427	1.492	0.892	0.568	0.709	0.635	0.917	0.789	0.744	0.649
	Ratio $\frac{\text{Vol. Exud.}}{\text{Vol. Abs.}}$	0.94	1.00	1.04	1.08	0.79	0.76	0.92	1.03	0.94	0.80	0.82	0.78
V	Absorption (mm.^{3})	0.211	0.293	0.552	0.601	0.762	1.006	1.830	2.223	1.931	1.391	0.842	0.763
	Exudation (mm.^{3})	0.195	0.260	0.373	0.503	0.616	0.905	1.590	2.178	1.924	1.441	0.681	0.600
	Ratio $\frac{\text{Vol. Exud.}}{\text{Vol. Abs.}}$	0.92	0.89	0.68	0.84	0.81	0.90	0.87	0.98	1.00	1.03	0.81	0.79

* Roots I, II, III, and IV were 5 days old and 44.8, 49.9, 52.3, and 51.1 mm. in length, respectively, when initial readings were made at 10 M.

excision, it may be that the bulb with developing leaves produced a water deficit in the root tissue. Water deficits and the requirements of growth might account for lower ratios during the first half of the 24-hour period before growth had ceased and before a ratio of 1.0 was attained. Root III, however, exhibited equal outflow and inflow during the second, third, and fourth intervals when growth took place and saturation deficits may have been present. Root III is also an exception to the fact that in most cases there is a marked increase in the magnitude of both inflow and outflow with time. Many young roots manifested a sixfold to tenfold or greater volume increase with time during the 24-hour experimental period. Exudation usually reached a maximum before the 24-hour experimental period ended. Whether or not this behavior indicates a diurnal cycle has not as yet been determined. During consecutive two-hour intervals a twofold and even a threefold change in volume exudation was sometimes observed.

Absorption and exudation of relatively apical segments

It was early observed that if an excised root was converted into an isolated segment by removal of the root cap region with a clean cut excision, exudation occurred solely at the basal end of the segment whether or not it was in an horizontal or a vertical position. When an excised root 65 mm. in length was cut in two, exudation appeared at both ends of the upper half. Individual experiments were made on 35 roots to determine the exact level at which exudation appeared at both apical and basal ends of an intermediate segment.

Measurements of simultaneous absorption and exudation were made on the intermediate segments which were isolated by removing different lengths of the apex and base of excised roots. The lengths of the tip removed differed by increments of 0.5 to 16 mm. Five potometer tubes were used; the cut ends of the intermediate segments extended into tubes 1 and 5, and water was absorbed from tubes 2, 3, and 4 placed between them. The potometers were 10 mm. apart. Owing to space limitations, data from only 9 roots are given in table II.

When less than 2 mm. was removed from the apical end of the excised root, the intermediate segment manifested elongation. This was due to the fact that the greatest amount of elongation in onion roots occurs in the second millimeter from the apex which in this case had not been removed. At the beginning of the experiment a small drop of water was placed over each cut end in potometers 1 and 5; this was usually absorbed by the apical end as shown by segments A, B, and C in roots I, II, and III (table II), which exhibited no exudation at the apical end. Segments with 5 to 16 mm. of the tip removed displayed absorption or exudation at the apical end during alternate intervals. The magnitude of total absorption, however, exceeded

TABLE II

SIMULTANEOUS VOLUME ABSORPTION AND VOLUME EXUDATION OF ISOLATED SEGMENTS MANIFESTING LITTLE OR NO EXUDATION AT THE APICAL END*

ROOT	AGE OF ROOT	TOTAL LENGTH OF ROOT	LENGTH OF BASAL SEGMENT REMOVED	LENGTH OF APICAL SEGMENT REMOVED	LENGTH AND DESIGNATION OF INTERMEDIATE SEGMENT IN POTOMETERS	TIME	LENGTH OF INTERVAL	POTOM. 1 VOLUME EXUDED AT APICAL END	POTOM. 1 VOLUME ABSORBED BY APICAL END	POTOM. 2 VOLUME ABSORBED	POTOM. 3 VOLUME ABSORBED	POTOM. 4 VOLUME ABSORBED	POTOM. 5 VOLUME EXUDED AT BASAL END	RATIO: VOLUME EXUDED / VOLUME ABSORBED
	days	mm.	mm.	mm.	mm.		hr.	mm.³	mm.³	mm.³	mm.³	mm.³	mm.³	
I	5	60.8	17	0.5	43.3 A	8 A.M. to 6:30 P.M.	10.5	0.0	0.093	0.648	0.277	0.740	1.370	0.71
						6:30 P.M. to 9 A.M.	14.5	0.0	0.0	2.830	1.295	3.328	2.330	0.98
II	5	63.0	18	2.0	43.0 B	8:05 A.M. to 6:35 P.M.	10.5	0.0	0.424	0.505	0.685	1.302	2.494	0.85
						6:35 P.M. to 9:05 A.M.	14.5	0.0	0.0	1.873	1.597	2.084	5.282	0.95
III	6	52.7	6	3.5	43.2 C	10:30 P.M. to 8 A.M.	9.5	0.0	0.091	0.603	0.603	1.043	1.972	0.84
						8 A.M. to 2 P.M.	6.0	0.0	0.617	0.522	0.782	0.929	2.852	1.00
						2 P.M. to 8 P.M.	6.0		0.782	1.043	1.108	1.304	4.322	1.02
IV	6	57.5	9	5.5	43.0 D	10:35 P.M. to 8 A.M.	9.5	0.124	0.0	0.630	0.456	0.815	1.114	0.65
						8 A.M. to 2:05 P.M.	6.0	0.0	0.685	0.300	0.489	0.980	2.208	0.90
						2:05 P.M. to 8 P.M.	6.0			0.326	0.489	0.815	1.543	0.95
V	7	70.1	20	7.5	42.6 E	8 A.M. to 1 P.M.	5.0	0.0	0.033	0.408	0.493	0.561	1.536	1.03
						1 P.M. to 6 P.M.	5.0	0.072	0.0	0.245	0.371	0.897	1.463	1.01
VI	5	69.1	16	10.0	43.1 F	8 A.M. to 4 P.M.	8.0	0.628	0.0	0.333	0.518	0.831	0.777	0.83
						4 P.M. to 8 P.M.	4.0	0.167	0.0	0.285	0.728	1.730	1.877	0.99
						8 P.M. to 10 A.M.	14.0		0.178	0.851	1.300	1.921	4.440	1.04
VII	5	64.7	9	12.5	43.2 G	3 P.M. to 8 P.M.	5.0	0.0	0.163	0.424	0.783	1.415	2.680	0.96
						8 P.M. to 8 A.M.	12.0	0.0	0.310	1.482	2.235	3.226	8.413	1.02
VIII	5	62.2	5	14.0	43.2 H	8:05 A.M. to 4:05 P.M.	8.0	0.098	0.0	0.627	0.764	0.894	1.821	0.83
						4:05 P.M. to 8:05 P.M.	4.0	0.0	0.390	0.185	0.813	0.975	2.398	1.04
						8:05 P.M. to 10:05 A.M.	14.0		0.169	0.715	2.760	2.760	6.328	1.00
IX	5	64.1	5	16.0	43.1 I	8:10 A.M. to 4:10 P.M.	8.0	0.146	0.0	0.540	0.390	0.683	1.434	0.98
						4:10 P.M. to 8:10 P.M.	4.0	0.0	0.065	0.390	0.358	0.943	1.450	0.84
						8:10 P.M. to 10:10 A.M.	14.0		1.010	1.430	1.50	2.940	6.850	0.98

* Experiments were carried out at 25° to 27° C.

that of total exudation in all but segments E and F from root V and VI. When more than 16 mm. was removed in roots of this age exudation usually exceeded absorption at the cut apical end.

The transport ratio of volume outflow to volume inflow $\frac{\text{total exudation}}{\text{total absorption}}$ was seldom unity during the first interval following excision. With time the ratio approached or reached unity, the isolated segment transporting all the water which was simultaneously absorbed; a ratio of one was not reached by growing segments. A comparison of the ratios during equal intervals of time and constant temperature as in segments C, D, and E from roots III, IV, and V in table II show that exudation may be equal to, slightly greater, or less than, absorption during consecutive intervals. In most cases in which the isolated segments were placed in the chambers the night before, the segments manifested oscillating ratios during two-hour intervals the following day but a ratio of unity when total outflow and inflow (sum of intervals) was compared. In table II, segments F, G, and H show ratios of one during the 12- or 14-hour period of the second or third interval; in this case, however, it is not known if the ratios were maintained from instant to instant.

The average rates of water intake from potometers 2, 3, and 4 were determined but are not given in table II owing to space limitations. The highest rates appeared at the relatively more basal levels and fluctuations of rates occurred changing the gradient of distribution from time to time. Comparisons of rates of water absorption of a given length of root when the root was intact (saturated atmosphere), when it had been excised, and finally when completely isolated from the root, showed that the given root tissue manifested the same type of behavior it exhibited before it was cut from the excised or intact root.

Direction of transport in isolated contiguous segments

Figure 1 is a diagrammatic representation of total outflow and of average rates of intake of water in three segments cut from a 78-mm. root 5 days old. The segments were placed in a vertical position with 5 horizontal potometers attached, two (designated by arrows 1 and 5, fig. 1) to collect exudate and three (designated by arrows 2, 3, and 4, fig. 1) filled with tap water. The highest average rate of intake appeared in the middle segment at a level 45 mm. from the apex. Comparison of apical and basal outflow in each segment showed that the direction of outflow in the apical third was entirely basal against gravity; in the middle and basal thirds outflow occurred in two directions; in both segments the basal outflow was greater. The total outflow in the apical segment was relatively low, but in this case not only were the rates of intake also lower but growth of the segment had taken place. Although the highest rate of intake occurred in the middle third, greater

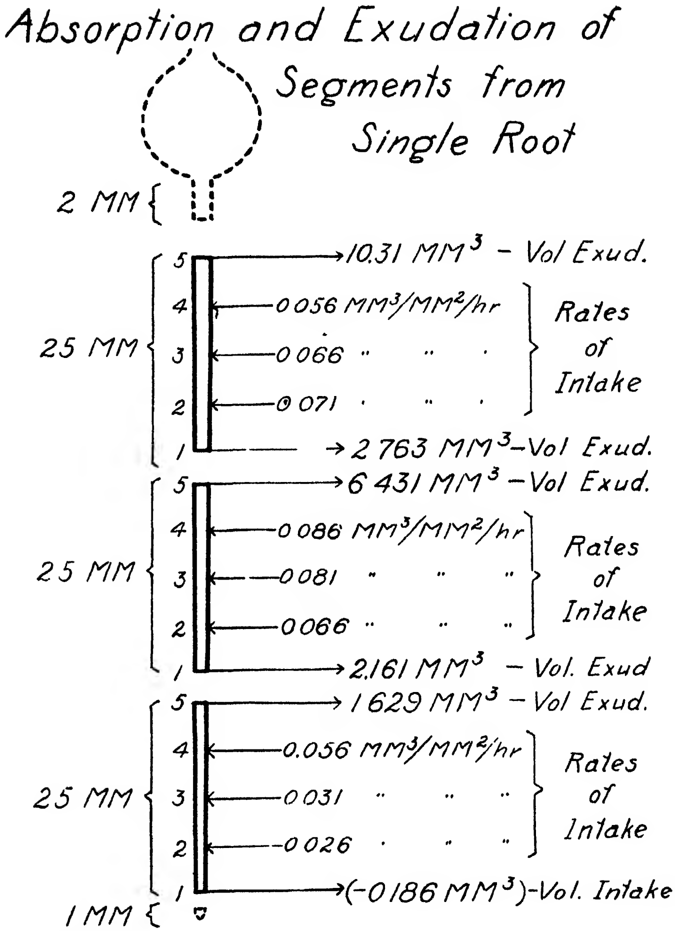

FIG. 1. Diagram of total volume outflow and average rates of intake of water in isolated contiguous segments of the same root. Positions of the potometers are designated by the arrows which are numbered 1, 2, 3, 4, and 5. Observations were made during a 20-hour period.

quantities of water were absorbed and transported by the basal third which was greater in diameter and, therefore, the area of absorption at the 3 potometers was correspondingly greater. The results obtained from this root are typical of segments of the same length and relative position cut from roots 75 to 100 mm. in length. Occasionally a basal segment exhibited greater exudation at the apical end of the segment but outflow occurred in both directions. When, however, basal segments were isolated from older and longer roots (over 200 mm. in length) and placed in their normal upright position with respect to gravity the direction of transport in the upright position was toward the apical end.

Figure 2 shows volume outflow of longer segments from two older roots designated as A and B. Observations were made during a 15-hour period with tap water in three potometers placed between those into which exudation flowed. In both roots, the relatively more basal segments manifested exudation at the apical end only. As indicated by the negative (–) sign most of the water covering the cut basal end disappeared. Since the vessels were open at both ends, this disappearance was caused by gravity, the water appearing in the potometer at the apical end in each case. In root A, no

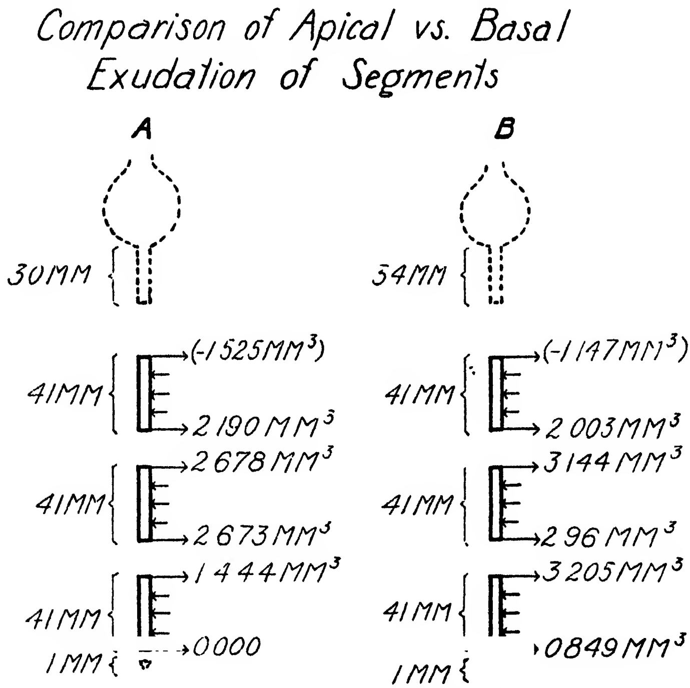

FIG. 2. Comparison of volume exudation at the ends of isolated contiguous segments of two roots designated as A and B. Positions of potometers designated by arrows.

exudation appeared at the apical end of the apical segment; root B, on the other hand, manifested a small quantity of exudation at this end. The second segment from the apex manifested exudation at both cut ends; the volume flow in each direction was equal in root A and practically so in root B.

The direction of outflow exhibited by the segments of the above two roots is characteristic of segments cut from older and longer roots. When basal segments were cut from roots 300 to 400 mm. in length and a drop of water was placed over each end, with the segment in the upright position, the water from the basal end flowed down the vessels appearing in the bottom (apical)

potometer immediately after; placed in an inverted position, the reverse was true; placed in a horizontal position equal amounts of exudation appeared in potometers at the two ends of the segments when water was absorbed from potometers in between.

The direction of transport through the stele of an isolated segment depends upon its age and upon the level of the segment relative to its position on the longitudinal axis. Segments from the apical third of young roots less than 65 mm. in length and less than a week old exhibit basal outflow only in an upright, inverted, or a horizontal position; segments from the middle and basal third exhibit outflow at both ends in all three positions. In isolated pieces of roots of this length the direction of transport is chiefly basal, structural features apparently playing a dominant rôle. If, however, segments are removed from levels near the bulb in roots over 200 mm. in length and 3 or more weeks old, the direction of transport depends upon the position of the isolated segment with respect to gravity. This indicates that the vector forces of transport are chiefly radial in isolated segments which are relatively older whereas in younger segments both longitudinal and radial factors are involved.

Discussion

It is important to note that the present investigation is concerned with *volume transport* and not pressures as such. Since external conditions were carefully controlled, the factors which brought about variations in the ratio of $\frac{\text{outflow}}{\text{inflow}}$ had their origin in changes in the root itself. That these factors are sharply localized is shown by the simultaneous changes in rates at the different levels of water intake. The total intake during any one interval is a summation of the local changes. Whether cells gain or loose water from one another is not shown but evidently the factors involved alter the flux relations of water in individual cells. This indicates the unequal distribution of *forces* (SHULL, 17) throughout the isolated root tissue under the given conditions. The axial gradient of intake at any one moment is the algebraic sum of individual cellular activities; it varies in relation to both time and space under the particular conditions employed. The data furnish no evidence to indicate whether or not the opposite variations of outflow and inflow are produced by separate mechanisms. It is not believed that they are produced by faulty technique; similar experiments, however, should be carried out by other investigators to establish this property of isolated roots.

With respect to water balance, determination of output, especially during short intervals, is not necessarily a measure of input and *vice versa.* This may be true in all types of roots; if so, conclusions regarding water absorption are not valid when based upon measurements of exudation and

not water intake itself. SPEIDEL (**19**) made parallel determinations of water intake and exudation in the decapitated root system of Plectranthus. He found that volume intake was greater than volume outflow throughout a 24-hour period. Similar results have been obtained with onion roots in a few experiments. In most cases, however, where intake was greater than outflow immediately after excision a ratio of unity was reached before 24 hours had passed.

The magnitude of both volume inflow and outflow increased with time after excision. It may be that the change in rates with time indicated an obscure periodicity. BARANETZKY (**1**) observed two-hour oscillations in the "bleeding" of decapitated root systems in *Ricinus insignia* plants 2 weeks old with an absence of decided periodicity at this age; but when observations were made on plants 5 weeks old, pronounced periodicity was noted.

Examination of GROSSENBACHER'S curves (**6**) obtained from topped Helianthus plants shows two- and threefold variations of rates of outflow between maxima and minima of 24-hour cycles under constant external conditions. Throughout a 12-hour period isolated onion roots frequently manifest a sixfold increase in rate of outflow. KRAMER (**11**) attached the cut stems of exuding cotton stem-root systems to a vacuum pump and noted (p. 486) that "four or five times as much water exuded under the reduced pressure as had exuded during the same period of time due to 'root pressure' alone." This change is no greater than that observed in a similar 12-hour period with onion root tissue in the absence of applied suction; in the onion root the change occurred under constant external conditions and was a property of the tissue itself; whether or not a similar capacity is present in cotton roots has not been shown. By attaching topped tomato root systems to a vacuum pump and lowering the pressure KRAMER (**12**, p. 787) "approximately doubled" the rate of exudation. Again it is interesting to note that single excised onion roots under constant conditions will do the same without artificially applied suction during consecutive two-hour intervals. KÖHNLEIN (**10**) and others have also applied suction to topped plants and obtained an increase in exudation; it may be that a similar increase in magnitude would have been manifested by the excised root tissue over a period of time under constant external conditions without the application of the suction pump. BREWIG (**3**), however, calls attention to the gradual decline of rate of exudation following excision of roots of *Vicia faba;* he maintains that in his experiments the rôle of "bleeding" can be ascertained only if measured immediately after cutting. When SPEIDEL made parallel measurements of water inflow and outflow in the decapitated root systems of Plectranthus he obtained a sharp increase in both, followed by a progressive decrease with time during a 24-hour period (**19**, fig. 23, p. 102). SPEIDEL attributes the steep descent of both outflow and inflow and the absence of

periodicity to oxygen deficiency produced by a gradual decrease of oxygen in the closed potometers. Closed potometers were not used in the present investigation.

JAMES and BAKER (8) described experiments with cut pieces of Sycamore roots which manifested uptake of water by the morphologically lower end and exudation at the upper end. They maintain that "the exudation is always in the morphologically upward direction even when the vessels have been opened at both ends, and will allow water to pass freely in the longitudinal direction. A drop of water placed at the physically upper end of the piece of root immediately causes a drop to appear at the lower end." Similar phenomena were not observed in pieces of onion root which manifested a longitudinal flow of water down the vessels when a drop of water was placed at the upper end of the root in a vertical position. Such pieces manifest exudation at either the distal or proximal end depending upon whether or not the segment is in an upright or inverted position; when oriented horizontally, exudation appears at both ends. JAMES and BAKER believed that their experiments furnished evidence that exudation from vessels did not take place. Although exudation from vertically placed basal segments cut from relatively old roots appeared in the lowest of a series of horizontally arranged potometers, the present experiments on onion roots do not furnish evidence for or against the possibility of phloem exudation since exudation from the phloem at the upper end of the segment would flow down the open vessels into the lowest potometer. The experiments do show, however, that epidermal absorption takes place in the absence of vessels filled with osmotically active solutes; forces outside of the vessels play a dominant rôle in the mechanism of intake in this case.

BREWIG (2, 3) maintains that variations of intake observed in the different root regions of *Vicia faba* are dependent upon transpiration. As mentioned earlier he passed air over portions of isolated roots and root segments to simulate transpiration. The experiments on the onion root show that fluctuations of intake in isolated roots occur in a saturated atmosphere under constant external conditions; in this case the regulatory phenomena are not associated with transpiration or evaporation from one end of the segment.

HEYL (7) has recently reviewed various theories dealing with the phenomena of "bleeding." He discusses theories which deal with tissue potentials, electro-endosmotic flow, varying conditions of the plasma membrane, unequal osmotic forces at opposite sides of the cell, variations in the distribution of osmotically active substances in the cell wall, rhythmic pulsation processes in living cells, variations in the osmotic suction strength of the vessel liquid, expansion and contraction of the cell or vessel walls, local differences in the osmotic pressure at different regions of a tissue, and changes

in pressure in the vessels as a result of injury. Whether or not one or more of the theories actually does apply to onion root tissue *per se* is only conjecture at the present stage of the work.

Summary

1. Simultaneous determination of water absorption and exudation of excised roots and pieces of roots revealed that fluctuations in both inflow and outflow occurred from one two-hour interval to another in a saturated atmosphere at a temperature which did not vary more than ± 0.5° C. during long intervals. The variations in outflow and inflow may be opposite in direction during any one interval.

2. The transport ratio of outflow to inflow, $\frac{\text{volume exudation}}{\text{volume absorption}}$, was seldom unity during the first interval following excision; with time the ratio approached or reached unity. Rates of both inflow and outflow usually increase with time during a 24-hour period following excision.

3. The direction of longitudinal transport through an isolated piece of root depends upon its age and upon its position on the longitudinal axis before cutting. Segments consisting of the apical third of young roots less than 70 mm. in length and less than a week old exhibit basal outflow only in an upright, inverted, or horizontal position with respect to gravity; segments from the middle and basal third exhibit outflow at both ends in all three positions. The direction of transport of basal segments cut from roots over 200 mm. in length and several weeks old depends upon the orientation of the segment with respect to gravity.

4. Isolated segments of the root manifest the longitudinal gradient of distribution of rates of absorption characteristic of the excised and intact state.

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AGENCIES AFFECTING THE PRODUCTION OF SUBSTANCE B BY *RHIZOPUS SUINUS*

CLAIR L. WORLEY

(WITH TWO FIGURES)

Introduction

In view of the wide distribution of growth factors[1] for microorganisms, it is not surprising to find that they can be obtained from other species of fungi and from bacteria, as well as from higher organisms. For many species there is no need to supply the factors, because they have the ability to synthesize these substances rapidly enough for optimal growth. Other species, although capable of manufacturing their own growth factors, do so at a suboptimal rate. In such cases, the deficiency of these substances becomes a limiting factor and their addition to the nutrient solution results in a marked acceleration of growth. Again, an organism may be totally unable to synthesize the needed compounds, and its growth, therefore, is strictly dependent upon the presence of these substances in the substratum. The amount of precise work that has been done on the classification of microorganisms into these three categories has been relatively large during the last decade. On the other hand, the quantity of work in relation to the agencies affecting the production of a growth factor, including vitamins, by the species of the first type mentioned, is very meagre and usually incidental to a larger problem.

NIELSEN (5) investigated the effect of the nitrogen source on the production of heteroauxin by *Rhizopus suinus*. Similar, though broader, tests were performed by BONNER (1) and by THIMANN (8). BOYSEN JENSEN (2) tested the effects of substrate, of temperature, and of pH on the heteroauxin production by *Aspergillus niger*. These constitute the known published papers that give consideration to physiological agencies affecting the production of growth factors by fungi. They are limited not only in number, but also in scope, and are concerned with the production of substance A (*i.e.*, heteroauxin). In no case have similar investigations been published relating to the production of substance B (an unknown substance increasing the dry weight production of *Aspergillus niger* and other species of fungi).

Procedures and methods

SPECIES EMPLOYED

The importance of the case history of all species of fungi used in physiological work has been mentioned frequently in the literature. It is now a

[1] "Growth factors" is used synonymously with growth substances, plant hormones, plant vitamins, growth accessory substances, etc.

well recognized fact that two fungi, isolated from the same culture, which show the same morphological and reproductive habits and are therefore classified as the same species, may respond in entirely different manners, physiologically. This physiological difference explains many of the discrepancies reported by various investigators working with the same taxonomic species.

A culture of *Rhizopus suinus* Niel. was obtained from stock cultures of the Botany Department of the University of Wisconsin. In preliminary tests, the fungus was cultured on NIELSEN's nutrient solution and under the conditions set forth by him. The filtrate was tested by the standardized pea, oat, and tomato-petiole methods for substance A, and by the *Aspergillus niger* method for substance B. In every case, positive qualitative results were obtained, and it was therefore concluded that this strain showed a physiological similarity to those employed by other workers. A transplant of *Aspergillus niger* was made from a stock culture maintained in the same Department. Preliminary tests showed that this strain was augmented by the Rhizopus filtrate but not by the Rhizopus cultural nutrient (see later). The strain of *Colletotrichum circinans* (Berk.) Vogl. was the one employed by WORLEY and DUGGAR (11) in an earlier investigation.

GENERAL PROCEDURES

The importance of details of technique in experimental work dealing with the growth factors of microorganisms has been emphasized in much of the literature. These are critically discussed in the reviews of PESKETT (7) and of KOSER and SAUNDERS (4). The main points listed in their reviews were considered in the conduction of the following series of tests. When dealing with the synthesis of an unknown substance by a new organism, however, the determination and the standardization of cultural techniques becomes an integral part of the investigations. Therefore, certain aspects of these cultural techniques are considered, one at a time, in the following investigations.

Throughout the entire investigation Pyrex glass containers were used. All apparatus was washed according to accepted methods and rinsed several times with distilled water, prior to use. The chemicals used were Baker's pure crystals, unless stated otherwise. And all cultures used as stocks for inoculation purposes were grown on solution III (see later). These stocks were cultured on slants in medium-sized test tubes, from which spores were washed with 20 ml. of physiological salt solution. The inoculum consisted of a 1-ml. aliquot of this preparation.

The standardized *A. niger* test method used is as follows. A 25-ml. portion of solution II and 5 ml. of the test solution were placed in each 125-ml. Erlenmeyer flask, in the method referred to as the 25:5 technique. In the 49:1 technique, 49 ml. and 1 ml. of the respective solutions were used. The

flasks containing the media were stoppered with cotton wadding and autoclaved for 15 minutes at 15 lb. pressure. After cooling, they were inoculated with an *A. niger* spore suspension according to the procedure already given. The inoculated cultures were grown at 31° ± 0.5° C., in diffused light, for 66 hours. This temperature was chosen because it proved to be the optimal temperature, as determined by preliminary tests. Since there is an increase in dry weight yield of *A. niger* until the 60th hour, and since there is a decline in the same caused by autolysis, after the 72nd hour, the mid-point, that is 66 hours, was chosen as the sampling time. At the termination of this time period the mats were filtered from the liquid, washed with distilled water, dried for three days in an electric oven at 92° to 96° C., cooled in a desiccator, and weighed.

The standardized *Rhizopus suinus* cultural method employed follows. Aliquots of 250 ml. of solution I were placed in liter Erlenmeyer flasks, stoppered with cotton, and autoclaved for 20 min. at 15 lb. pressure. After cooling, they were inoculated with a *R. suinus* spore suspension according to the given procedure. These cultures were grown at 25° ± 1° C., in diffused light, for 5 days. At the end of the cultural period the mats were separated from their substrata by filtration; the filtrates were autoclaved and stored for the Aspergillus tests, while the mats were washed, oven dried, cooled, and weighed. This general method was the basic cultural technique from which variations were made. In each test, one influencing agent is varied, and this variance is given with each test.

Nutrient solutions

The following nutrient solutions were used throughout this series of tests:

Solution I (for Rhizopus cultures)

NH_4-tartrate	10.228 gm.	(M/18)
$MgSO_4 \cdot 7H_2O$	0.493 gm.	(M/500)
KH_2PO_4	0.545 gm.	(M/250)
Dextrose	10.005 gm.	(M/18)
Ferric tartrate (sat. soln.)	3.000 drops	
Dist. H_2O to 1000 ml.		

Solution II (for Aspergillus cultures)

NH_4NO_3	5.003 gm.	(M/16)
$MgSO_4 \cdot 7H_2O$	0.616 gm.	(M/400)
KH_2PO_4	0.619 gm.	(M/220)
Sucrose	62.215 gm.	(M/5.5)
Ferric tartrate (sat. soln.)	3.000 drops	
Dist. H_2O to 1000 ml.		

Solution III (for slants and for Colletotrichum plates)

NH_4NO_3	5.003 gm. (M/16)
$MgSO_4 \cdot 7H_2O$	0.616 gm. (M/400)
KH_2PO_4	0.619 gm. (M/220)
Dextrose	20.010 gm. (M/9)
Ferric tartrate (sat. soln.)	3.000 drops
Difco agar	17.000 gm.
Dist. H_2O to 1000 ml.	

Statistical analyses

During this investigation, which extended over a period of two and one-half years, the productivity of the strain of *R. suinus* slowly and progressively decreased. Thus, while the figures obtained for any one specific test are strictly comparable, this is not necessarily true for the figures of different tests. It seemed inadvisable, therefore, to average replicas and take their probable errors, as followed by the majority of workers in related fields. In lieu of the heretofore accepted procedure the following statistical method was employed. All measurements made, regardless of type, were recorded for the replicas of both controls and tests. Ratios, that is the test value divided by the control value, were calculated for every possible combination. The total number of ratios possible is the product of the number of test values and the number of control values. These ratios were averaged to obtain the value of the statistical factor (F) and its standard error (SE) was determined. This method of assay has two distinct advantages over the methods in common usage: (1) it readily permits the comparison of two different sets of data; and (2) the standard error obtained is greatly enlarged by extremes in test and control values and therefore is of greater significance. This is especially true when its value is relatively small.

Results

I. The influence of the weight of the Rhizopus mat on the production of substance B

The Rhizopus was cultured for five days in 250 ml. of solution I placed in 1-liter flasks. These were held at a temperature of 23° ± 1° C., while all other runs were cultured at 25° ± 1° C. The former were grown in the presence of a bottom light source obtained from six 100-watt Mazdas, placed at a distance of 16 inches and separated from the flasks by a glass plate and a one-inch running-water filter. Four filtrates of the Rhizopus cultures were tested by the Aspergillus method at three concentrations.

The results in table I fail to show any correlation between the amount of the growth factor produced and the amount of Rhizopus mycelium con-

TABLE I

Rhizopus suinus: THE RELATION OF THE DRY WEIGHT OF THE MYCELIUM TO THE PRODUCTION OF SUBSTANCE B (3 REPLICAS)

CULTURE	RHIZOPUS: DRY WEIGHT OF MYCELIUM	CONCENTRATIONS TESTED TS*: NUTRIENT: H_2O	ASPERGILLUS: DRY WEIGHT OF MYCELIUM (AVERAGE)	F ± SE
	mg.		*mg.*	
Control	....	0: 40: 10	35.8	1.00
		1: 40: 9	64.1	1.79 ± 0.024
Test filtrate I	111.0	5: 40: 5	67.9	1.90 ± 0.014
		10: 40: 0	97.0	2.71 ± 0.022
		1: 40: 9	42.6	1.19 ± 0.013
Test filtrate II	108.0	5: 40: 5	78.5	2.19 ± 0.019
		10: 40: 0	119.2	3.33 ± 0.037
		1: 40: 9	42.6	1.19 ± 0.035
Test filtrate III	180.4	5: 40: 5	68.4	1.91 ± 0.018
		10: 40: 0	125.9	3.51 ± 0.024
		1: 40: 9	38.9	1.09 ± 0.012
Test filtrate IV	189.0	5: 40: 5	64.8	1.81 ± 0.047
		10: 40: 0	81.9	2.29 ± 0.053

* TS = test solutions; *i.e.*, the Rhizopus filtrate.

tributing to its production. In each case, however, the yield of *A. niger* was increased with an increase in the amount of the filtrate tested. The above test was repeated twice using the same culture flasks in the same positions. The results were very comparable and showed the same interrelationships as those included in table I. These data suggested that environmental agencies might exert important rôles on the productivity of the stimulant. And since the distribution of the light was not exactly even, this test was repeated in total darkness, in order to investigate this aspect more thoroughly.

II. THE INFLUENCE OF THE WEIGHT OF THE RHIZOPUS MAT ON THE PRODUCTION OF THE GROWTH FACTOR, WHEN CULTURED IN TOTAL DARKNESS

The method of culturing the Rhizopus was the same as that used for test I, save that the culturing took place in total darkness and at 25° ± 1° C. All the filtrates were tested by the standard 25: 5 Aspergillus technique.

These results, like those of test I, fail to show any relationship between the amount of fungal growth and the substance B production. Once again, marked variations occurred between the stimulated powers of several Rhizopus cultures. These variations were repeatable in the same order and degree by using the same flasks in the same positions. Test II tends to eliminate light as the causal agent in the environment. These variations may be caused by a balance between the productions of growth promoting

TABLE II

Rhizopus suinus: The relation of the dry weight of mycelium, for cultures grown in total darkness, to the production of substance B (5 replicas)

Culture	Rhizopus: dry weight of mycelium	Aspergillus: average dry weight of mycelium	F ± SE
	mg.	*mg.*	
Control	..	105.3	1.00
TS-1	49.7	170.0	1.62 ± 0.014
TS-2	53.1	135.1	1.29 ± 0.009
TS-3	63.7	177.4	1.70 ± 0.004
TS-4	49.3	185.2	1.76 ± 0.013

and growth inhibiting factors. The succeeding tests investigate the first hypothesis, and arguments in support of the second hypothesis are presented throughout this investigation and the one to follow (which includes physico-chemical properties of the active factors in the filtrate). The non-correlation noted in the above two tests (I and II) is in accord with the production of substance A by the same species of fungus (Bonner, **1**). These results suggested the advisability of separating substances A and B prior to testing.

III. The effect of various concentrations of an ether-treated filtrate of Rhizopus, on the dry weight yield of *A. niger*

The Rhizopus was cultured in accordance with the standard method given above. The mat was filtered from the liquid, and the filtrate was layered three times (15 minutes) with equal volumes of peroxide-free ether. Hetero-auxin was demonstrated in the ether-soluble fraction by the accepted pea and oats methods. The aqueous portion, containing ether-insoluble compounds, was tested by the standard *A. niger* method.

TABLE III

Dry weight yields of Aspergillus on various concentrations of ether-insoluble filtrates (TS) of *Rhizopus suinus* (5 replicas)

Culture	Concentrations tested TS: Nutrient: H_2O	Aspergillus: average dry weight of mycelium	F ± SE
	ml.	*mg.*	
Control	0: 40: 10	38.0	1.00
TS-1	1: 49: 9	76.8	2.23 ± 0.032
TS-2	2: 40: 8	84.3	2.46 ± 0.068
TS-3	5: 40: 5	115.5	3.36 ± 0.028
TS-4	10: 40: 0	104.3	3.03 ± 0.030

Test III confirms the work of Nielsen and Hartelius (**6**) by which it was demonstrated that the Rhizopus filtrate actually contains two growth

factors which may be separated by their respective solubilities in ether; *viz.*, substance A, or heteroauxin, which is ether soluble; and the ether-insoluble substance B which augments the production of dry matter by *A. niger.*

It is obvious that 5 ml. of the ether-insoluble fraction of the filtrate, in 50 ml. of the test nutrient, yields the maximal results. With the use of untreated filtrate (*cf.* table I), however, much greater concentrations are required before the maximal yield is reached. Since a greater variation in the concentration remains more nearly parallel with the subsequent yield with the use of the untreated filtrate, as compared to the ether-insoluble fraction, the former was used throughout the following tests. Subsequent tests, to be reported later, indicate that the stimulant might be a labile complex and not one definite compound. Therefore, until the definite physicochemical properties of the so-called substance B are known, the untreated filtrate is used in the tests, since any pre-treatment might produce an indeterminable artifact.

IV. The influence of the liquid area exposed per unit volume on the production of substance B by *R. suinus*

The Rhizopus was cultured in various sized containers so that the area exposed, for 50 ml. of nutrient, varied from 16 sq. cm. to over ten times that area. The inoculum was in proportion to that of the standard method (*i.e.*, ⅓ the customary amount), and all other conditions were held as heretofore. The various filtrates were tested by the 25:5 Aspergillus method.

TABLE IV

Influence of area exposed per unit volume on the production of substance B (7 replicas)

Culture expressed as area exposed	Rhizopus: dry weight of mycelium	Aspergillus: average dry weight of mycelium	F ± SE
sq. cm.	*mg.*	*mg.*	
Control		247.5	1.00
162.0	133.1	317.1	1.29 ± 0.056
136.9	97.9	270.2	1.10 ± 0.017
99.0	68.5	260.4	1.06 ± 0.016
83.8	42.5	298.9	1.22 ± 0.029
49.5	80.4	351.1	1.43 ± 0.019
31.3	37.0	320.1	1.30 ± 0.017
24.3	33.7	352.5	1.43 ± 0.022
16.0	39.4	290.4	1.17 ± 0.018

The data in table IV fail to reveal any relationship between the areas exposed per unit volume and the effectiveness of the filtrates. These results have two possible interpretations. First, the relative size of the surface area plays no part in the production of substance B. Secondly, the surface area

influences not only the production of substance B, but also of a substance which inhibits the growth of *A. niger.* As a result, the effectiveness of the filtrate would not depend solely on the amount of substance B but rather on the balance of this substance with the inhibitory factors produced in the same culture. The second possibility is the more plausible. It is well known that the presence of inhibitors in substrata on which fungi are cultured is more universal than the occurrence of stimulators. Furthermore, the production of heteroauxin by *R. suinus* parallels the degree to which the cultures are aerated, or to the relative area exposed [BONNER (1) and THIMANN (8)]. The depression of heteroauxin on the growth of the strain of *A. niger* used was demonstrated by qualitative tests. Heteroauxin was significantly toxic to the strain of *A. niger* employed in concentrations as low as 1 p.p.m., and markedly so at 10 and 100 p.p.m. This is within the range of heteroauxin occurring in *Rhizopus suinus* filtrates. It is, therefore, entirely possible that the surface area did influence the production of substance B, but also affected, in like manner, the production of inhibitors (heteroauxin ?). The untreated filtrates, therefore, failed to show a relationship between the effectiveness and the area exposed, because the stimulating value did not depend on the growth factor alone, but on the balance of this with inhibitory products. Prior to the complete solution of this problem, as well as of other problems relevant to growth factor production, it will be necessary to separate the stimulators from the inhibitors.

V. EFFECT OF WHITE LIGHT ON THE PRODUCTION OF SUBSTANCE B BY *Rhizopus suinus*

Three Rhizopus cultures were assembled and grown under standard methods, with the following variances. Two flasks were so placed for growth as to be subjected to a bottom white light with an energy value of 570 to 580 ergs/cm.^{2}/sec. The third flask was cultured under similar conditions except that it was kept in total darkness. The respective filtrates were tested by the standard 25 : 5 Aspergillus technique.

TABLE V

EFFECT OF LIGHT AND DARKNESS ON SUBSTANCE B PRODUCTION (3 REPLICAS)

CULTURE	RHIZOPUS: DRY WEIGHT OF MYCELIUM	ASPERGILLUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE
	mg.	*mg.*	
Control	....	270.3	1.00
Darkness	99.0	292.4	1.08 ± 0.007
Light 1	32.6	285.0	1.05 ± 0.011
Light 2	96.6	270.3	1.00 ± 0.009

The results in table V indicate little or no effect of bottom light on the production of this growth factor. It is to be noted that one of the cultures exposed to the light produced a filtrate which was ineffective, and that the other produced a filtrate which was less effective than that normally experienced. A comparison of the Rhizopus dry weight yields and the respective values of their filtrates emphasizes once again the lack of correlation between actual growth and the production of the growth factor.

VI. Effect of monochromatic irradiations on substance B production

When employing a light from a top source it is necessary to prevent a focusing effect from the container and any shading from the cotton plugs. This was accomplished by using 12-oz., flat-sided, reagent bottles, which were placed on the broad side under the light whose effect was to be tested. The controls were arranged in a similar position, but placed in total darkness. Because of the size of the containers it was necessary to use 50 ml., rather than 250 ml., of the culture nutrient. All filtrates were tested by the 25:5 technique.

The three monochromatic lights were arranged and standardized by Dr. William Gray, to whom sincere thanks are extended. The ultimate light source was a mercury lamp and the monochromatic lights were obtained by combinations of four liquid dye solutions, each 1 cm. in depth. Those used were:

Green .... Main line at 5640 Å. Intensity 224 to 232 ergs/cm.^{2}/sec. Filter layers: 0.22 per cent. potassium dichromate; 6 per cent. $CuSO_4$; and two of neodymium-NH_4NO_2.

Blue .... Main line at 4360 Å. Intensity 288 ergs/cm.^{2}/sec. Filter layers: 2 per cent. quinine-HCl; 6 per cent. $CuSO_4$; 0.003 per cent. acid-rhodamine; and ammoniacal-$CuSO_4$.

Yellow .... Main line at 5780 Å. Intensity 392 ergs/cm.^{2}/sec. Filter layers: 6 per cent. $CuSO_4$; 2 per cent. quinine-HCl; 0.02 per cent. tartrazine + 0.02 per cent. erythrosine; and distilled H_2O.

The effects of the monochromatic lights on the production of substance B are more marked than the effect of the entire visible range. This difference, perhaps, is the result of the direction of the incident light; the white light striking the under side of the mat, and the others the top surface. A more plausible explanation lies in the possibility of opposite effects of the various light ranges tending to balance each other in the complete visible range. This is indicated by the data of table VI. The yellow light greatly reduced the effectiveness of the Rhizopus filtrate as compared with the control cultures in the dark, while the green and the blue lights had the opposite effect.

TABLE VI

EFFECT OF MONOCHROMATIC BLUE, GREEN, AND YELLOW LIGHTS ON THE PRODUCTION OF SUBSTANCE B BY *B. suinus* (7 REPLICAS FOR YELLOW, AND BLUE, 3 REPLICAS FOR GREEN)

CULTURE	RHIZOPUS: DRY WEIGHT OF MYCELIUM	ASPERGILLUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE
	mg.	*mg.*	
Control 1	... ...	228.3	1.00
YELLOW light			
Test 1	15.8	411.1	1.78 ± 0.026
Test 2	22.2	379.0	1.65 ± 0.023
Dark 1	55.2	582.7	2.55 ± 0.108
Dark 2	45.3	487.2	2.14 ± 0.023
BLUE light			
Test 1	31.0	491.4	2.16 ± 0.019
Test 2	26.0	493.7	2.18 ± 0.025
Dark 1	43.4	461.9	2.03 ± 0.028
Dark 2	40.5	442.0	1.94 ± 0.101
Control 2	....	270.3	1.00
GREEN light			
Test 1	49.1	335.4	1.24 ± 0.021
Test 2	42.0	324.4	1.20 ± 0.011
Dark 1	97.9	299.5	1.11 ± 0.013
Dark 2	80.3	293.1	1.08 ± 0.007

Whether the lights exerted their influences directly on the filtrate or indirectly on the mycelium is not known. All cultures grown in total darkness produced mycelia completely submerged. Those grown in the presence of the lights, however, did develop floating mats with typical aerial hyphae. There was neither sporulation nor pigmentation when subjected to either yellow or blue light. When subjected to green light the sporulation was comparable to that of the white light tests and the pigmentation was but slightly less intense. In these tests, however, there was no direct relationship between the type of growth and the growth factor productivity.

VII. INFLUENCE OF THE SIZE OF THE RHIZOPUS INOCULUM ON THE ULTIMATE PRODUCTION OF SUBSTANCE B

Although the size of the Rhizopus inoculum was varied in neither preceding nor subsequent tests, and therefore played no part in the variations recorded there, it seemed advisable to ascertain whether it influenced the substance B productivity of the cultures. Standard Rhizopus cultures were used except that the size of the inoculum was varied from one-eighth to twice the amount employed in the standard technique.

The data of table VII show that a doubling of the size of the Rhizopus inoculum does result in a significant increase in the stimulative power of the ultimate filtrate. Since the amount of the physiological solution used was

TABLE VII

EFFECT OF THE SIZE OF THE INOCULUM ON THE SUBSTANCE B PRODUCTION BY *Rhizopus suinus* (7 REPLICAS)

CULTURE EXPRESSED AS SIZE OF THE INOCULUM	RHIZOPUS: DRY WEIGHT OF MYCELIUM	ASPERGILLUS: AVERAGE DRY WEIGHT OF MYCELIUM	$F \pm SE$
	mg.	*mg.*	
Control	......	356.5	1.00
2 normal	47.0	525.7	1.48 ± 0.013
Normal	54.7	462.1	1.30 ± 0.007
½ normal	48.6	448.2	1.26 ± 0.007
¼ normal	60.0	425.1	1.19 ± 0.007
⅛ normal	51.3	408.1	1.15 ± 0.007

the same for all tests, the inorganic ions thus introduced were not responsible for the results obtained. There are two possibilities of carry-over effects; that in the liquid, and that in the spores themselves. That the former of those two is most probably not the causal agent is indicated indirectly by the fact that increases of Rhizopus filtrates, many fold greater, exert lesser degrees of acceleration. The possibility of a carry-over effect in the spores was not tested. The most acceptable possibility, however, is an indirect one through the effect of the size of the inoculum on the subsequent Rhizopus growth. The greater the concentration of the inoculum, the greater the rate of the initial growth, the earlier the sporulation, and the earlier the initiation of the subsequent autolysis. If substance B production is correlated with the rapidity of growth, with spore production, or with autolysis, this latter hypothesis would tend to explain the results of table VII above. Throughout this series of investigations it was observed that the stimulative power of the filtrate was always greatest in those cultures in which the Rhizopus had formed spores previous to the termination of the run. And, at present, it is tentatively assumed that the last explanation is the most plausible for the interpretation of the results obtained.

VIII. THE RELATIVE PRODUCTIVITY OF SUBSTANCE B, BY *Rhizopus suinus*, AT DIFFERENT TEMPERATURES

Seven standard cultures of Rhizopus were grown at various temperatures. They were placed in available incubators, baths, and rooms whose temperatures fluctuated less than 1° C. The temperatures used were: 11°, 18°, 25°, 31°, 35°, and 41° C. All other conditions were held as nearly equal as possible. The relative humidities and the intensities of the diffused lights, however, varied slightly. The test at 11° C. failed to grow and consequently was not tested. The filtrates of the remaining six cultures were tested by the standard 25 : 5 Aspergillus technique.

TABLE VIII

EFFECT OF TEMPERATURE ON THE SUBSTANCE B PRODUCTIVITY OF *Rhizopus suinus* (3 REPLICAS)

CULTURE EXPRESSED AS TEMPERATURE	RHIZOPUS: DRY WEIGHT OF MYCELIUM	ASPERGILLUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE
	mg.	*mg.*	
Control	. .	270.3	1.00
18° C.	88.9	279.5	1.04 ± 0.032
25° C.	109.2	283.7	1.05 ± 0.007
28° C.	200.0	300.5	1.11 ± 0.031
31° C.	771.3	422.3	1.56 ± 0.007
35° C.	128.2	300.0	1.11 ± 0.017
41° C.	43.2	230.4	0.85 ± 0.007

It is obvious that the production of substance B is affected markedly by the incident temperature. Whether this effect is direct or indirect has not been ascertained. Up to and including the optimal temperature of 31° C. there is a relationship between the weight of the Rhizopus mat and the effectiveness of the filtrate. The relationship between the productivity and the abundance of aerial hyphae and sporulation is even more marked. These in outline form are:

18° C.—floating mat; no aerial hyphae; no spores.
25° C.—floating mat; aerial hyphae; few spores.
28° C.—floating mat; aerial hyphae; good spore production.
31° C.—floating mat; aerial hyphae reaching 1 inch in height; very intense sporulation, evident as early as 3½ days.
35° C.—floating mat; aerial hyphae; good spore production.
41° C.—submerged gelatinous mass.

The rapidity with which the mycelia developed was correlated with the degree of sporulation. This, once again, indicates the possibility of a relationship of the production of this growth factor either with the rate of growth or with the process of sporulation. The results obtained at 41° C., however, would require a separate explanation.

NIELSEN (5) reported that the maximal yield of substance A (heteroauxin), by the same species, occurred at 35° C. And, BOYSEN JENSEN, investigating the production of heteroauxin by *Aspergillus niger* (2), recorded that the greatest accumulation occurred at 36 to 37° C. Their results suggest that the sudden drop in effectiveness noted for the filtrates from cultures grown at temperatures above 31° C. might possibly be due to a marked increase in the production of substance A. The effectiveness tested, therefore, could be the result of the balance of substances A and B. The inhibiting effect of the filtrate of the 41° C. culture is more probably caused by some metabolic product other than heteroauxin, since the production of this

substance is known to be very low at the higher temperatures. Although the results obtained are not necessarily indicative of the absolute productivity of substance B, they do show that the stimulative value of the Rhizopus filtrate, as tested by *Aspergillus niger*, is influenced markedly by the temperature. The cardinal temperatures, in relation to the effectiveness of the filtrate, are: minimal, near 18° C.; optimal, 31° C.; maximal, slightly above 35° C. It is assumed, however, that the production of substance B parallels this effectiveness relatively closely.

IX. Aeration of the Rhizopus culture and its effect on the substance B production

Six standard Rhizopus cultures were used for these tests. One was plugged with cotton wadding as used throughout all other tests (solution K). The second flask was closed with a rubber stopper and sealed with paraffin (solution Seal). The third culture was aerated with sterilized air bubbling through the solution at the rate of six bubbles per minute (solution Air). The fourth was the same as the third, save nitrogen was substituted for the air (solution N). In the fifth, pure oxygen replaced the air of the third (solution O–1). And in the sixth, the space above the solution was aerated with pure oxygen at the same rate (solution O–2). Other cultural procedures were as of the standard technique, and the untreated filtrates were tested by the 25:5 method.

TABLE IX

Effect of aeration on substance B production (3 replicas)

Culture	Rhizopus: dry weight of mycelium	Aspergillus: average dry weight of mycelium	F ± SE
	mg.	mg.	
Control*	....	100.8	1.00
Seal†	144.0	145.5	1.45 ± 0.028
K*	149.1	160.2	1.59 ± 0.015
O–2	300.2	170.2	1.65 ± 0.043
Air	173.7	174.1	1.73 ± 0.056
O–1	174.5	206.5	2.06 ± 0.045
N	No growth	....	....

* Four replicas.
† Submerged mat.

The production of substance B is directly related to the degree of aeration, as is illustrated by the results shown. This increase in substance B accumulation, resulting from increased aeration, is undoubtedly more marked than the above data indicate, since Bonner (1) showed that heteroauxin production, by the same species, is greatly increased by an increase in aerobic metabolism, and since preliminary tests demonstrated the toxicity of this substance to the growth of *Aspergillus niger*.

The results obtained for the sealed culture show that the flask contained ample air, not only for growth but for a significant production of substance B. Although, in most cases, the production of this growth factor appeared to possess a relationship with rapid initial growth and with spore formation, these same results demonstrate that the process of asexual fruiting is not absolutely essential. A continuous exchange of the air above the liquid (O–2) resulted in a greater effectiveness of the filtrate, while the stimulative value of the filtrate of the flask stoppered with cotton wadding was intermediate to these last two. Herein lies a partial explanation of the variations in effectiveness observed for various cultures grown under strictly standard methods. The tighter the wadding the less the degree of gaseous exchange, and therefore a decrease in the production of the growth factor. Complete aeration of the liquid and of the gas above it (O–1) resulted in a 50 per cent. increase in production over that obtained for the sealed culture. Since the Rhizopus failed to grow under anaerobic conditions, and since growth is a prerequisite to substance B production it was impossible to ascertain whether oxygen is essential for the formation of this growth factor.

The data above, once again, demonstrate the lack of a relationship between the mycelial production of Rhizopus and the amount of the substance B produced.

X. Effect of filter paper on the production of substance B by *Rhizopus suinus*

It has been reported that filter paper not only influences the accumulation of the heteroauxin formed, but also that it stimulates the growth of this fungus (Worley, **10**). It seemed advisable, therefore, to test whether filter paper would play a similar rôle in substance B production. Two flasks of the fungus were cultured; the one was run under standard culture methods, while the other contained one shredded 120-mm. filter paper. The filtrates were concentrated to one fifth their original volumes, by partial evaporation, and then diluted to the concentrations desired. The following concentrations were prepared and tested.

F–5.0 = 1 ml. of concentrate equivalent to 5.0 ml. of filter-paper filtrate.
F–2.5 = 1 ml. of concentrate equivalent to 2.5 ml. of filter-paper filtrate.
etc.
W–5.0 = 1 ml. of concentrate equivalent to 5.0 ml. of regular filtrate.
W–2.5 = 1 ml. of concentrate equivalent to 2.5 ml. of regular filtrate.
etc.

The filtrates were tested not only by the *Aspergillus niger* method (table XI), but also by the *Colletotrichum circinans* method reported by Worley and Duggar (**11**). In lieu of the diameter measurements, as given in their paper, the comparisons were made on total areas (table X). This change

permits a comparison with dry weight measurements of fungi grown on liquid cultures. The results are also given in a form to show the relative effects of the test solutions at different time periods. In addition to the two accepted methods of interpretation, *viz.*, radial, and ring-area, the sector-area method was employed. The advantages of this latter method have been given by WORLEY (9).

TABLE X

EFFECT OF FILTER PAPER ON SUBSTANCE B PRODUCTION, AS TESTED BY THE *Colletotrichum circinans* METHOD (5 REPLICAS)

CULTURE	TOTAL AREAS (SQ. CM.) OF *Colletotrichum circinans* AT VARIOUS TIME INTERVALS							
	0	3 DAYS	6 DAYS	8 DAYS	11 DAYS	14 DAYS	17 DAYS	20 DAYS
Control	0.008	0.33	2.75	5.30	10.18	15.55	22.90	31.47
F–5.0	0.008	0.61	3.73	7.65	13.85	22.06	32.17	42.78
F–2.5	0.008	0.55	3.63	6.33	13.20	21.24	30.58	41.06
F–1.5	0.008	0.52	3.56	6.20	12.32	19.24	27.81	37.18
F–1.0	0.008	0.57	3.63	6.29	12.76	19.87	28.27	38.26
F–0.5	0.008	0.65	3.70	6.68	12.88	19.01	27.34	35.26
W–5.0	0.008	0.54	3.56	6.25	12.25	19.48	28.56	38.48
W–2.5	0.008	0.40	2.52	4.83	10.24	17.20	26.15	35.78
W–1.5	0.008	0.31	2.81	5.68	11.70	18.70	28.37	37.50
W–1.0	0.008	0.54	3.37	5.94	12.38	19.32	27.25	37.39
W–0.5	0.008	0.54	3.43	6.16	12.44	19.32	25.97	33.29

The same test solutions were diluted to one part in five and then tested by the 25 : 5 *Aspergillus niger* method. The results are given in table XI.

TABLE XI

EFFECT OF FILTER PAPER ON SUBSTANCE B PRODUCTION, AS TESTED BY *Aspergillus niger* (3 REPLICAS)

CULTURE	ASPERGILLUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE
	mg.	
Control	52.3	1.00
F–2.5	217.0	4.16 ± 0.062
F–1.5	207.9	3.98 ± 0.068
F–1.0	184.2	3.53 ± 0.055
F–0.5	138.4	2.65 ± 0.149
W–2.5	362.3	6.94 ± 0.219
W–1.5	231.8	4.44 ± 0.086
W–1.0	192.9	3.69 ± 0.061
W–0.5	173.8	3.31 ± 0.044

The interpretation of the effect of filter paper, in the nutrient solution, on the production of substance B is dependent upon the test organism used. The above data show a significantly higher augmentation of the growth of *Colletotrichum circinans* on the addition of a filter-paper filtrate than on

the addition of a regular filtrate. This increase in acceleration, arising from the presence of the filter paper, is significantly greater regardless of the concentration of the filtrate used. It is of interest to note that the lower concentrations of the test filtrate frequently show a greater augmentation during the earlier stages of growth, while increasing amounts give parallel increases of growth during the later stages. Throughout these tests, however, the regular filtrate (without filter paper) tended to show a lesser stimulation than the other at the same concentration. That a tenfold concentration did not show an increase in growth ten times as great, and the fact that the less concentrated filtrates show a greater acceleration during the earlier stages are indicative of other factors being limiting in effect on the utilization of superoptimal amounts of substance B by *Colletotrichum circinans*. The results of the higher concentrations are given in graphic form (fig. 1) to

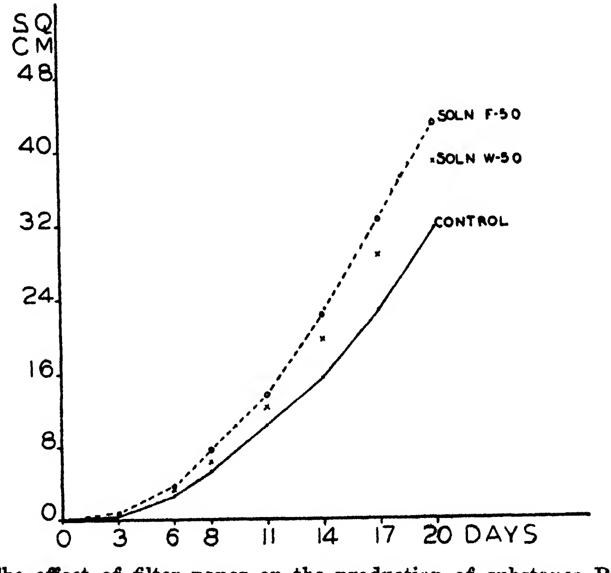

FIG. 1. The effect of filter paper on the production of substance B, as tested by *Colletotrichum circinans* (discussion in text).

show more clearly the difference of effects between the regular and the filter-paper filtrates upon this organism. Both the data of table X and of figure 1 indicate a steady acceleration of the tests over the control throughout the entire duration of the runs. That the above is not the case is shown by the calculations of ratios, one set of which is given in graphic form as figure 2. It becomes obvious that the growths of the test cultures, as compared with the control culture, are very erratic, and that the more stimulative the filtrate the greater is its variability. Most of the tests show a marked stimulating effect during the first three days, an inhibiting one during the next several,

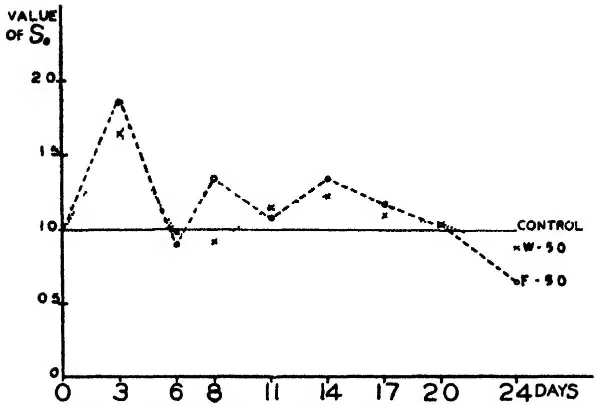

FIG. 2. The irregularity of stimulation at various time intervals (discussion in text).

a second and lesser augmentation up to approximately the seventeenth day, no effect near the twentieth day, and a slight inhibition thereafter. No explanation is offered for these marked variations in the degrees of acceleration during the various periods of growth.

These same factors were tested by a second organism, *viz.*, *Aspergillus niger*. The data of table XII reveal that the effectiveness of the test filtrates upon this fungus was the reverse of that shown by *Colletotrichum circinans*. In this case, the regular filtrates showed a marked and consistently higher stimulating effect than those of the filter paper.

This difference in effectiveness, as shown by the two test organisms, is further proof of the presence of both inhibiting and stimulating factors. It is also possible that the two test organisms are stimulated by two different metabolic products. Such an explanation, however, is not plausible. This is also shown by recent work which indicates a greater and greater similarity of growth factors for the various microorganisms. It is tentatively assumed that the effectiveness is dependent upon the balance of stimulators and inhibitors, and that a given balance may affect two test organisms in different manners. If it is assumed that the inhibitor and the stimulator mutually influence *Aspergillus niger* when present in the same concentrations, then the inhibitor (heteroauxin ?) must accumulate in greater concentrations in the presence of filter paper than does the growth factor. If this assumption is true, the effect of the factor on *Colletotrichum circinans* must be more potent than the inhibitor. The results with *Aspergillus niger* alone could be interpreted as showing that filter paper augments the accumulation of an inhibitor and plays a lesser or no part in the accumulation of the factor. The results with *Colletotrichum circinans* are then indicative of different growth regulators or are proof that the above mentioned condition does not exist. On the other hand, if there are but two substances, and if the effec-

tiveness of these two contrasting factors are different reciprocally, their respective accumulations cannot be hypothesized from the data at hand. In any case, however, a given balance of the two contrasting factors must affect the two organisms in different manners. A fourth explanation is possible. The filter paper itself may contain a soluble substance stimulative to *Colletotrichum circinans* and another inhibitive to *Aspergillus niger*. These substances may have been carried over in the filtrates and thus exert an effect on the organisms. The concentration of any soluble substance of the filter paper in the ultimate test solution, however, would be so small that this possibility is of little value. Furthermore, all solutions were filtered with the same type of filter paper, thereby permitting the accumulations of any such hypothetical substances in both types of test filtrates. Regardless of the explanation accepted, the first being the more plausible, the addition of filter paper results in an increased acceleration for *Colletotrichum circinans* and a decreased augmentation for *Aspergillus niger*.

General discussion and summary

Few studies in the metabolism of microorganisms have dealt with the quantitative production of the so-called growth factors. Such investigations have been limited by the lack of sufficiently specific tests. Until these factors have been isolated or at least freed from concurrently occurring inhibitors and other stimulators, and until either biological or chemical tests have been developed to measure a specific compound microquantitatively, it will be impossible to ascertain the effect of the environment or the composition of the substratum on the quantitative production of growth factors by these lower organisms. Therefore, present studies are *nothing more than indications in induced alterations in the effectiveness* of a solution or tissue. They result in but a poor insight into the changes in effectiveness and fail to prove definitely whether the alteration is due to a change in the absolute amount of the growth factor or to a change in the balance between this factor (and other stimulators) and the inhibitors. Throughout this discussion it is to be remembered that the effects stated are those on effectiveness rather than on substance B itself.

A survey of all tests run demonstrates the lack of a correlation between the amount of the growth factor produced (*i.e.*, total effectiveness) and the amount of *Rhizopus suinus* mycelia contributing to its production. Certain tests, however, do indicate a relationship of substance B production with the type of growth. Either a rapid initial growth or an intense spore production by the Rhizopus results in a greater accumulation of the factor in its filtrate. Although most tests show a relationship between sporulation and substance B production, other results show that the fructifications are not a prerequisite for the formation of the factor. It is also possible that these

two types of growth are indicative of an early autolysis, and that it is this latter process which liberates the active principle into the liquid substratum.

No relationship exists between the areas exposed per unit volume and the effectiveness of the respective filtrates, even though the surface areas vary over a tenfold range. Since the work of others shows that the production of heteroauxin by *Rhizopus suinus* is dependent upon the surface area, and since this substance acts as an inhibitor to the test organism, it is plausible that the production of substance B is affected in like manner and that the constant effectiveness of the filtrates indicate rather the lack of change in the balance between stimulators and inhibitors.

White light exerts little or no effect on substance B formation. The effects of monochromatic lights, on the other hand, were more marked. Yellow light reduces greatly the effectiveness of the Rhizopus filtrate, while green and blue lights have the opposite effect. No tests were conducted to determine whether these effects were direct or indirect through the type of growth induced.

A doubling of the size of the Rhizopus inoculum resulted in a significant increase in the effectiveness of the ultimate filtrate. This effect is most probably due to a more rapid initial growth followed by early sporulation and subsequent autolysis.

Changes in temperature have the most marked effect of any agent tried. The cardinal temperatures for the production of this factor are: minimal, near 18° C.; optimal, 31° C.; maximal, 35+° C. These effects are probably indirect through the type of growth or are the result of the effective balance of substance B and heteroauxin productions.

One set of tests demonstrated that the production of substance B is directly related to the degree of aeration.

One run tested the effect of filter paper on the growth factor accumulation. If tested by *Colletotrichum circinans* there was an acceleration in the production, while if tested by *Aspergillus niger* there was a slight decrease. Four possible explanations were given.

The variations noted for replicas of Rhizopus control cultures are possibly due to additive effects of very slight variations in size of the inoculum, degree of aeration (*i.e.*, tightness of wadding), the incident light, and the temperature.

Sincere thanks and appreciation are extended to Dr. B. M. Duggar, of the University of Wisconsin, under whom this work was conducted and the protocol of this paper prepared.

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EFFECTS OF pH AND THE COMPONENTS OF BICARBONATE AND PHOSPHATE BUFFERED SOLUTIONS ON THE METABOLISM OF POTATO DISCS AND THEIR ABILITY TO ABSORB IONS[1]

F. C. Steward and C. Preston

(WITH NINE FIGURES)

Introduction

This paper consists of two parts. The first concerns the effect of certain variables on the absorption of bromide by potato discs and experiments which were made by the senior author in the Division of Plant Nutrition, University of California, in 1933–1934. The second part describes the effect of similar treatments on the metabolism of potato discs and experiments carried out with the assistance of the junior author at Birkbeck College, London, in 1937–1938. Since the results of the later work are essential for a full interpretation of that which preceded it, the two investigations are described together.

Investigation of the effects of external pH presents an obvious approach to the problem of salt accumulation. It is, therefore, an essential part of any survey of the variables which affect this important process (**18, 19, 20**). Despite the great emphasis which others have placed upon the rôle of pH in salt absorption, detailed discussion of the effect of this variable on bromide absorption by potato discs has been long postponed. For some preliminary observations see (**18**). This delay was due to the knowledge that until the effects of a wide range of variables—including the nature and concentration of neutral salts—upon the behavior of cells had been investigated, phenomena due to hydrogen ion *per se* could not be segregated from those due to other variable components of the system used to control pH. Knowledge of the metabolic processes of potato tissue and the way these are affected by neutral salts (**21, 22, 23**) is now adequate to permit investigation of the problem.

Of the buffer systems which can be used to regulate the pH of solutions in contact with plant cells, that which depends upon the proportion of bicarbonate to free carbonic acid is of outstanding interest since its components are metabolic products of cells. This paper contains a survey of the effects of pH, carbon dioxide, and potassium carbonate concentration. Though somewhat incomplete, it enables us to visualize the trend of the effect of these variables upon absorption and metabolism of potato discs and prepares

[1] This paper is the fourth of a series of papers on the biochemistry of salt absorption by plants. The writers are indebted to Prof. D. R. Hoagland for proofreading this paper.

the way for the rather formidable task which a still more complete investigation entails. A similar survey made with phosphate—buffered solutions shows the extent to which the effects obtained in bicarbonate solutions are peculiar to a particular buffer system.

Methods

Potato discs were used under the standard conditions which have proved conducive to salt accumulation. Bromide was used as an indicator of anion absorption and was supplied as potassium bromide at the same equivalent concentration throughout a series of experiments. All known variables, other than salt concentration and pH, which affect salt absorption were standardized as follows: temperature 23° C., mean disc thickness, 0.75 mm, number of discs in two liters of solution which was stirred (100 r.p.m.) and aerated (total gas flow 15 liters per hour) to maintain equilibrium with the oxygen tension in the gas used. This facilitated comparisons between a series of experiments which, though not run concurrently, were carried out in rapid succession using tissue from the same uniform stock of tubers. A standard experimental time of 70 or 72 hours was used.

The experiments were of two general kinds: experiments at constant salt (potassium bicarbonate or phosphate) concentration but embracing a range of pH values; and experiments at constant pH in which the total salt (potassium bicarbonate or phosphate) was the variable. At constant potassium bicarbonate concentration, the range of pH values was obtained by changing the partial pressure (composition by volume at constant pressure) of carbon dioxide in the gas stream which flowed through the culture vessels. For the comparable procedure in the phosphate series free acid (H_2SO_4 or H_3PO_4) was added to solutions of potassium phosphate (K_2HPO_4) as required. To reveal the specific effects due to these buffer salts (potassium bicarbonate and phosphate) without confusion with those due to pH, the total salt concentration was varied at *constant pH*. To eliminate confusion between the effects due to the free acid or anion and those merely due to the cation (K), certain experiments were also carried out at constant potassium concentration. In the latter case a low concentration of the experimental salt (potassium bicarbonate or phosphate) was raised to the desired potassium level by the addition of the required amount of a salt (potassium sulphate) in which the anion has been shown (22) to be without conspicuous effects on the metabolism of potato discs.

The required partial pressure of carbon dioxide was obtained by combining streams of oxygen and carbon dioxide at known rates of flow. Oxygen was used in order that even the richest mixture (26.6 per cent. CO_2) did not contain an oxygen pressure so low that behavior of the tissue would be limited thereby. Manifolds were connected to a high pressure source of

oxygen which delivered the *total* flow required at a constant rate which was regulated by a reducing valve. A similar device was also used for carbon dioxide. For each gas mixture, the flow of oxygen and carbon dioxide, drawn off at convenient points on the manifolds, was regulated by separate needle valves which were adjusted in accordance with the readings of a calibrated flow meter (**15**) through which the mixture passed. Thorough mixing occurred in the large pressure stabilizers which were described with the original apparatus (**15**). The final adjustment of the needle valves was so made that the gas mixture passing through a wash bottle which contained the required bicarbonate concentration, gave the requisite pH as seen by the indicator which was added for the purpose. The final record of the pH of the salt solutions actually in contact with the tissue was made by a glass electrode, but the indicator in the wash bottle gave warning if the mixing apparatus was not working faithfully. In this way the reaction of solutions was kept within narrow limits throughout the period of experiments. The volume percentage of carbon dioxide in the gas mixture used was recorded by gas analysis in a modified type of HEMPEL's apparatus.

Results

THE EFFECT OF pH, POTASSIUM BICARBONATE, AND DISSOLVED CARBON DIOXIDE ON BROMIDE ABSORPTION

The combined results of three experiments are assembled in table I. The choice of the concurrent treatments was determined partly by convenience in operation and partly by considerations which need not concern us here; it is the impression which the *combined* results convey of the effect of pH, added bicarbonate, and carbon dioxide on the accumulation of bromide which is of interest.

At constant bromide concentration, in the absence of added bicarbonate and in solutions in equilibrium with a gas free of carbon dioxide, the bromide uptake was not significantly affected by the extreme range of total potassium concentration which these experiments incurred. This factor can thus be ignored. It is very clear, however, that the components of the system which did affect bromide absorption profoundly were the concentration of hydrogen ions, bicarbonate, and carbon dioxide. It remains to be seen which of these is the most important.

At any given pH (pH 7.23 and 6.8) the effect of a simultaneous increase of bicarbonate and dissolved carbon dioxide is to greatly reduce the bromide absorbed; so much so, in fact, that by such increases alone the *accumulation* of this ion *was almost entirely suppressed* even though all other variables were favorable. Ignoring for the moment the differences in pH (7.2 and 6.8) at which two different experiments were conducted, it can be shown that over the range of added bicarbonate concentrations (0.0 to 0.020 equiv.

TABLE I

EFFECT OF pH, [$KHCO_3$] AND [CO_2] ON ACCUMULATION OF BROMIDE BY POTATO DISCS FROM 0.001 EQUIVALENTS PER LITER KBr AT 23° C. DURING 70 HOURS

EXPERIMENT*	[$KHCO_3$] EQUIV. PER LITER	PERCENTAGE CO_2†	MEAN pH OF EXTERNAL SOLUTION	[Br] SAP. PER LITER	EXPERIMENT	[$KHCO_3$] PER LITER	PERCENTAGE CO_2†	MEAN pH OF EXTERNAL SOLUTION	[Br] SAP. PER LITER	EXPERIMENT	[$KHCO_3$] PER LITER	PERCENTAGE CO_2†	MEAN pH OF EXTERNAL SOLUTION	[Br] SAP. PER LITER
		%		*mg. equiv.*		*mg.equiv.*	%		*mg. equiv.*		*mg.equiv.*	%		*mg. equiv.*
1	–	26.6	4.75	7.88	1	0.001	26.6	5.3	5.26	1	–	0.0	6.90	17.3
										3	–	0.0	7.70	17.3
2	–	7.8	5.92	14.3	3	0.001		6.8	15.3	2	0.0050	4.2	7.23	11.5
										2	0.0100	7.8	7.23	6.8
2	–	4.2	6.12	16.1	2	0.001	0.8	7.3	15.8	3	0.0025	2.2	6.81	14.1
2	–	0.8	6.58	16.5	3	0.001		7.7	15.9	3	0.0150	12.5	6.76	2.9
1	–	0.0	6.90	17.3	1	0.001	0.0	8.3	16.1	3	0.0200	16.5	6.81	1.7
3	–	0.0	7.70	17.3										. ..

* Expt. 1. Total [K] = 0.002 equiv. per liter
Expt. 2. Total [K] = 0.011 " " "
Expt. 3. Total [K] = 0.021 " " "
† Volume percentage CO_2 in gas phase.

per liter) the relationship between bromide absorbed by the discs and either the external potassium bicarbonate concentration (fig. 1) or the volume percentage of carbon dioxide in the gas phase, is smooth. At pH 7.2 and 6.8 the curves are almost coincident and are linear over much of the range of bicarbonate (0.0 to 0.010 equiv. per liter) or carbon dioxide concentrations (0 to 8.0 per cent. by vol.) although they do deviate at higher bicarbonate concentrations from the straight line as they tend to approach somewhat asymptotically the bromide concentration of the external solution. It is, therefore, clear that some component of the system which is increased proportionally to the added bicarbonate or the dissolved carbon dioxide depresses the bromide uptake. To fully map out the depressant effect of bicarbonate and dissolved carbon dioxide at each pH value would be a considerable task. It suffices for present purposes, however, to identify by a straight line the slope of the curve which expresses this relationship and, extrapolating this to zero bromide concentration, slight error will be encountered only at the highest concentrations of bicarbonate.

In the absence of added bicarbonate, increased pressure of carbon dioxide in the gas phase caused lower pH in the solution; when it became more acid than pH 6.9, the bromide absorption was decreased—especially so below a critical value of approximately pH 6.0—when the decrease was rapid (fig. 1). As shown by paired cultures which received the same gas mixture, the

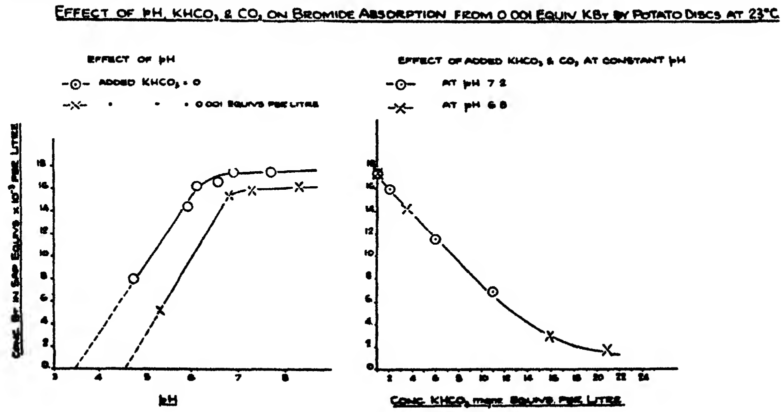

FIG. 1.

dissolved carbon dioxide, free to exert its unbuffered effect on pH, depressed bromide absorption much less than did the combined effect of dissolved carbon dioxide and added bicarbonate at more alkaline reactions. To appreciate the full range of effects of bromide absorption (70 hr. at 23° C. from 0.001 equivalents KBr per liter), the effect of pH and, at each pH, of increased bicarbonate and carbon dioxide concentration must be visualized. This can be done if three co-ordinates (bromide concentration in sap after

70 hr., pH of external solution, bicarbonate concentration in the solution or percentage by volume of carbon dioxide in the gas) are plotted simultaneously (fig. 2). This method of presentation will be used throughout, as it permits the effects of several variables to be visualized simultaneously.

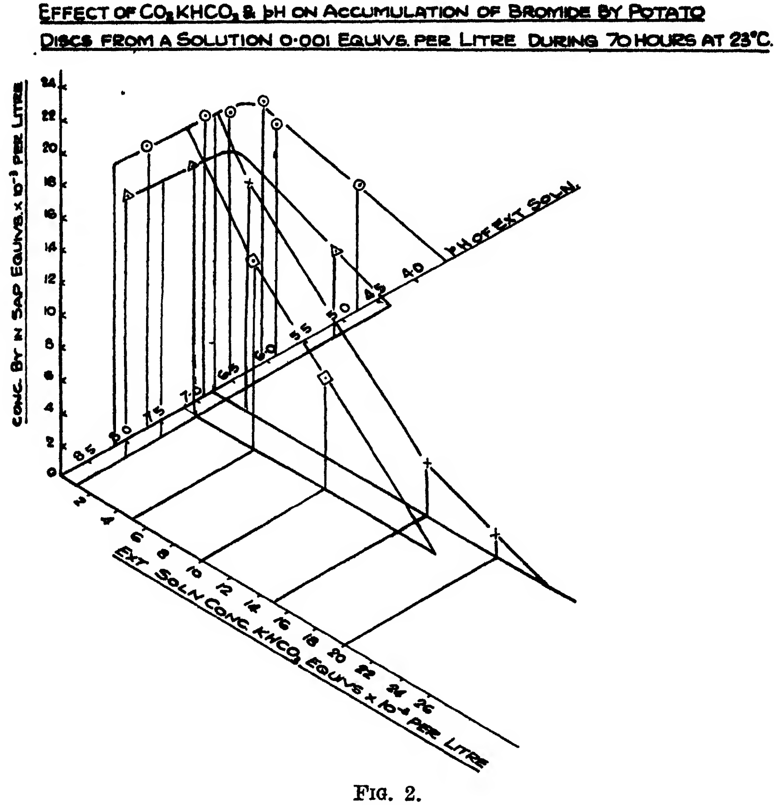

FIG. 2.

To specify completely the relationships of bromide uptake to pH in bicarbonate buffered solutions the surface of a solid model is required. By making the fullest use of the data available, it is possible to visualize the kind of surface which expresses these relationships. The solid model may be constructed in two different ways and each serves a useful purpose.

THE EFFECT OF PH AND ADDED $KHCO_3$ ON BROMIDE UPTAKE.—In the simplest procedure, the two variables plotted are those which were set at arbitrary values in the actual experiments; namely, pH, and the concentration of potassium bicarbonate added to the solution. The third is the concentration of bromide observed in the sap after a 70-hour treatment. One surface of such a model (fig. 3) can be specified at once from the data at zero concentration of added potassium bicarbonate. At each pH, a section

through the model parallel to the bicarbonate concentration axis is bounded by the line, assumed for this purpose straight, which records the rate of *decrease* of bromide uptake with increased bicarbonate and carbon dioxide concentration. A sufficient number of these lines enable the surface of the complete model to be visualized.

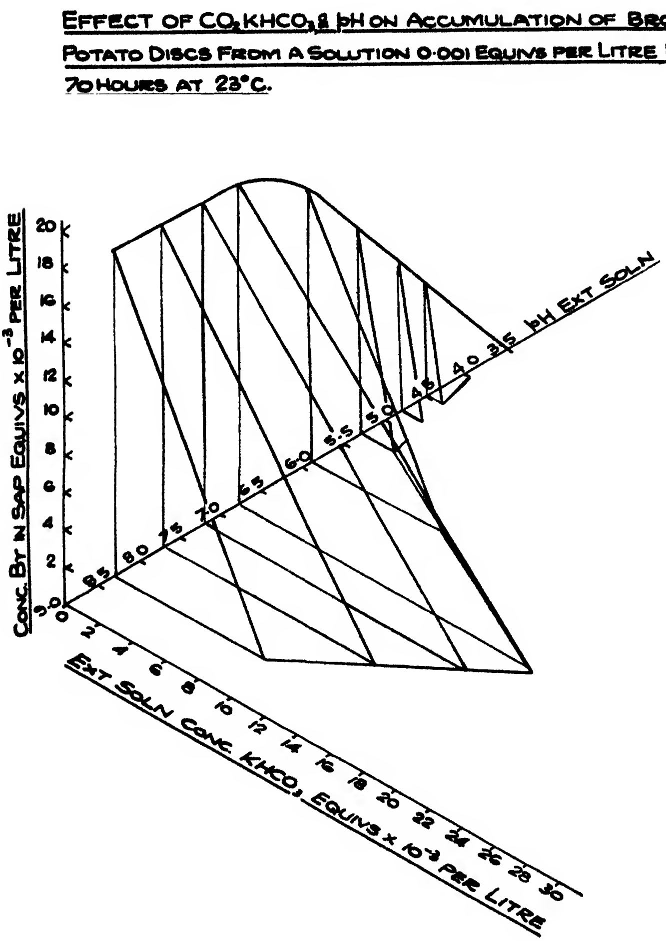

FIG. 3.

Figure 3 shows that, in the absence of added bicarbonate there is a considerable range (pH 8.0 to 6.0) in which pH and dissolved carbon dioxide concentration have but little effect on bromide absorption. That a hundredfold range of hydrogen ion concentration is without conspicuous effect on bromide uptake is in agreement with other results (**6**, **18**, p. 1012) which show that salt absorption may be less dependent upon pH than upon other

variables. At reactions more acid than pH 6.0, bromide absorption was reduced and its decline with acidity implies that at *about pH 3.5 bromide absorption should vanish* (fig. 3). At increased bicarbonate concentration (0.001 equiv. per liter) bromide uptake was decreased at all reactions (table I; figs. 2, 3) and from the graph (fig. 2) it is clear that bromide uptake should vanish at a much less acid reaction (pH 4.5) in presence of 0.001 equivalents of bicarbonate.

Having fixed the reactions (pH 3.5 and 4.5) at which bromide uptake vanishes at zero concentration of bicarbonate and at 0.001 equivalents per liter, it can be estimated with sufficient accuracy for the intermediate concentrations. The bromide concentration attained in the sap from solutions at pH 5.3 and two different bicarbonate concentrations can be found, and these values fix the steep slope of the bromide uptake-bicarbonate concentration curve which intercepts the plane of zero bromide uptake (fig. 2) at a bicarbonate concentration of 0.002 equivalents per liter. At pH 6.8 and 7.2 the data available fix the slope of the curve which represents the effect of bicarbonate and carbon dioxide on bromide uptake (fig. 2) with some certainty; and by joining points at which bromide absorption vanishes, the probable boundary of the model is thus ascertained. At each of the two reactions (pH 7.7 and 8.3) data on bromide uptake are available for one bicarbonate concentration only. It can be obtained, however, at the other by reference to the well defined curves of figure 2. Although these two points represent the minimum to establish the slope of the line passing through them, these lines have been prolonged in figure 3 to the plane of zero bromide uptake. It will be shown later that the resultant figure and the curve in which it cuts the plane at zero uptake—a curve which specifies the conditions of pH and bicarbonate concentration which suppress bromide absorption—can be correlated with the metabolism of potato discs under a similar set of conditions.

The characteristics of this model which are of interest are as follows: At constant pH the depression in the bromide absorption which is due to an increase in potassium bicarbonate concentration is a minimum at a pH of 6.8; *i.e.*, uptake is at a maximum. This reaction is near to strict neutrality and also to the pK value of carbonic acid as a monobasic acid. At pH = pK, (pH = 6.4) the ratio $\frac{\text{salt}}{\text{free acid}} = 1$. At reactions more acid than this the amount of free acid becomes rapidly much greater than the salt added; at reactions more alkaline than pH = 6.4 the free acid decreases until at pH 8.3 it is virtually zero. Since there is a greater effect of added bicarbonate at constant pH at *both* more acid and more alkaline reactions than pH = 6.4, the effective component of the system cannot be immediately selected from all those which are affected by increased bicarbonate concentration. It is

suggestive that at acid reactions where the unneutralized carbonic acid exceeds the salt present, the effect of increased bicarbonate concentration at constant pH is particularly great.

THE EFFECT OF pH AND TOTAL CARBONIC ACID ON BROMIDE ABSORPTION.—In figure 4, the variables plotted are bromide concentration in the sap (mg. equiv. per liter), pH of the external solution, and total carbonic acid in the solution; *i.e.*, that which is present as salt ($KHCO_3$) *plus* the uncombined acid. For the bicarbonate solutions the free acid can be calculated from the relation $\text{pH} = \text{pK}_1 + \log \frac{[\text{salt}]}{[\text{free acid}]}$ where $\text{pK}_1 = 6.4$ and in the absence of potassium bicarbonate the total dissolved carbonic acid is given by the relationship: mols H_2CO_3 per liter $= 3.645 \times 10^{-2} \times P$, where P = partial pressure in atmospheres (volume percentage of $CO_2 \times$ total barometric pressure in atmospheres) of carbon dioxide in the gas phase. The data are given in table II.

In figure 4, data from experiments at the same concentration of *added*

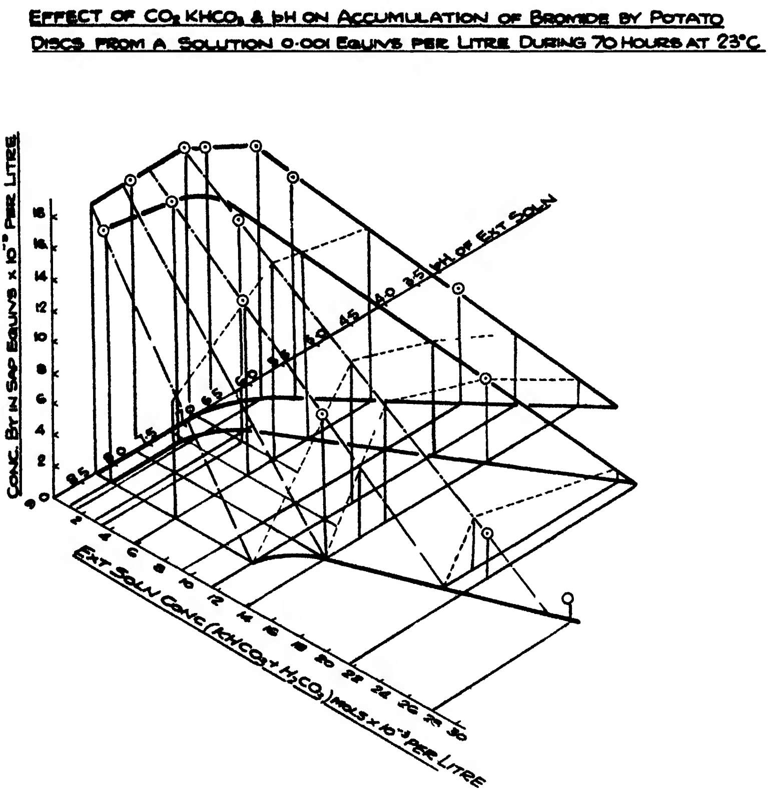

FIG. 4.

potassium bicarbonate all lie in a curved surface which cuts the basal plane of the model in a curve. This curve which traces out the increase in the total carbonic acid as a given concentration of bicarbonate (initially at pH 8.3), is brought to more acid reactions by dissolved carbon dioxide. Such a curve, at zero added potassium bicarbonate, defines one boundary of the model; another, at 0.001 equivalents of added $KHCO_3$ per liter, has been drawn. At points on these curves, indicated by the thin construction lines, ordinates have been erected which correspond with the experimentally observed bromide concentration in the sap. The several data at pH 7.2 and 6.8, respectively, define two vertical sections cut through the model at these pH values[2] and the edge of the section thus exposed (which can be treated as linear) represents the *depressing effect at constant pH of total dissolved carbonic acid on bromide uptake.* In this way two curves and two straight lines, which lie in the surface of the model, can be defined. At any given *total* carbonic acid concentration vertical sections[3] can be cut through the model (at right angles to the sections at constant pH) and ordinates erected to intercept the curves which lie in the surface and have been defined above. Observed data at pH 7.5 and 8.3 were insufficient to establish the section along these planes of constant pH value but, by prolonging to pH 7.5 and 8.3 the slope of the lines which connect points in the surface of the model at the same total acid concentration, the experimental data can be supplemented; thus sections through the model at these pH values may be established with sufficient accuracy for present purposes.

This second model now corrects a possible misconception gained from the first. Both models show that at pH 8.3 (where free carbonic acid can be neglected) the depressing effect of added bicarbonate (total carbonic acid present in the system) on bromide uptake is great. This effect must be due either to $[HCO_3^-]$ or to this supplemented by $[OH^-]$ since the only free undissociated acid is that which arises from hydrolysis. The effect of increased concentration upon bromide absorption, *per unit of total carbonic acid present,* at *constant pH,* is a maximum at pH 8.3. It decreases progressively at more acid reactions at which relatively more of the acid present is in the uncombined and undissociated form. This must mean that, as between bicarbonate ion and undissociated free acid, the *most effective component of the bicarbonate buffer system which suppresses the uptake of bromide is the bicarbonate ion itself.* It is equally clear, however, that other factors are involved. Were it not so, all of the data could be fitted to a smooth curve of bromide uptake plotted against bicarbonate ion concentration and this is not possible. Solutions which are acid due to carbon

[2] Where such sections cut the surface of the model is shown by a chain-dotted line in the figure (.–.–.–).

[3] Where such sections cut the surface of the model is shown by a broken line in the figure (––––––––).

dioxide, unbuffered by potassium bicarbonate, exert on bromide uptake a retarding effect which is out of all proportion to the bicarbonate ion which they contain; this suggests that the free carbonic acid also has its specific effects on the bromide absorbed—either due to the undissociated free acid or the H^+ *per se.*

In the absence of added bicarbonate the bromide uptake is but little affected by increased concentration of dissolved carbon dioxide between pH 7.7 and 5.9; but at more acid reactions it is depressed and, in the pH range 7.7 to 4.7, the data fall on a straight line, the empirical equation of which is $[Br] = 17.3 - \frac{17.3}{17.0} \times [\text{dissolved } CO_2]$ where the concentrations of bromide in the sap and carbon dioxide in the solution are expressed in milligram equivalents and millimols per liter, respectively.

In the presence of added bicarbonate the effect of increased carbon dioxide on bromide accumulation is due to the combined action of the bicarbonate and the free acid. Although the concentration of salt and free acid is either known or can be calculated at the given pH values, the data are hardly adequate to derive a satisfactory empirical relationship between dissolved carbon dioxide, bicarbonate, pH or $[H^+]$, and bromide absorption which covers the full range of conditions. Such a relationship could not do more than describe concisely the models shown—it would not alone explain the effects of bicarbonate and carbon dioxide on bromide uptake, since these factors clearly operate through the metabolic processes which are involved in absorption and which have yet to be described.

It happens that all of the data in tables I and II lie on a smooth curve of bromide uptake plotted against total carbonic acid in the external solution. This relation is not general, however, and would probably not hold at high concentrations of total carbonic acid and reactions more alkaline than pH 7.7.

At any given pH value, a simultaneous increase of potassium bicarbonate and dissolved carbon dioxide retarded, and eventually suppressed, bromide uptake. Both salt (bicarbonate ion) and free undissociated acid (H_2CO_3) contributed to this effect which was clearly not due to the H and OH ions. If the effect of bicarbonate is additive to that of carbonic acid, then simple calculations show that at constant pH, near neutrality, the drop in bromide concentration in the sap due to the external bicarbonate was 1.1 milligram equivalents per liter of sap per milligram equivalent of bicarbonate in 1 liter of external solution.

The retardation of bromide uptake which was due to increase of potassium bicarbonate concentration at constant pH is the more interesting because increased concentrations of other potassium salts (with anions Cl, Br, NO_3) stimulate all those processes which are concerned in salt accumu-

lation (**22**). Therefore, the processes which are deemed essential to salt uptake should be suppressed by increased concentrations of total carbonic acid at constant pH and evidence to be described shows that this is, in fact, the case.

Specific effects of pH on bromide uptake.—An obvious difficulty is to ascertain from the evidence whether H^+ and OH^- have any direct effect upon bromide uptake. In solutions enriched with bicarbonate, the bromide uptake falls off more at alkaline reactions than near neutrality and this result is evident from the models (figs. 3 and 4) whether one compares cultures of constant potassium bicarbonate concentration or of the same *total* carbonic acid concentration. If the bromide uptake was influenced only by the bicarbonate ion and the free acid then, since their relative effects are almost equal at pH 7.0, the effect of increased carbonic acid in the system should be independent of pH provided the *total* (bicarbonate and dissolved carbon dioxide) remained constant. Figure 4 shows that such is not the case. At reactions more acid than pH 5.9 and more alkaline than 7.2, bromide uptake is less than at the intermediate reactions; this effect becomes the more conspicuous as the total capacity of the tissue to absorb is retarded by carbon dioxide and bicarbonate (see vertical section through model of figure 4 along 0.014×10^{-3} mols total CO_2 per liter). Such effects obtained in the more acid and more alkaline reactions *are attributable only to acidity and alkalinity per se* and, therefore, to specific effects of H^+ and OH^-. This statement is made only after a full attempt to account for the effects observed *solely* on the basis of the calculated concentrations of bicarbonate ion and undissociated carbonic acid without reference to H^+ and OH^-.

There is thus *a range, relatively broad in the absence of bicarbonate, within which bromide uptake is not much affected by hydrogen ion concentration and its attendant variables. Reactions near to neutrality, however, are at any given total concentration of added carbonic acid, more favorable to bromide uptake than either more acid or more alkaline solutions.* The investigation of the effects of carbonic acid and bicarbonates on metabolism shows that metabolic processes now known to be closely associated with bromide uptake are similarly affected and show optima near pH 7.0. The view that these are, in fact, effects due to H and OH ions is strengthened because similar results have been obtained in solutions of other buffer salts; *e.g.*, phosphates.

These data predict the acid reactions at which bromide uptake should vanish. In solutions unbuffered by bicarbonate there are no data above pH 8.3. The form of the models suggests that at still more alkaline reactions bromide uptake might also decline and eventually vanish. This problem, upon which it is interesting to speculate, can be solved only if experiments are carried out with other buffer systems, or in a range of pH where the second dissociation of carbonic acid operates.

TABLE II

MOLS OF ADDED BICARBONATE AND TOTAL ACID (COMBINED AND UNCOMBINED H_2CO_3) IN SOLUTIONS USED

pH	Added $KHCO_3$	Total acid	pH	Added $KHCO_3$	Total acid	pH	Added $KHCO_3$	Total acid	pH	Added $KHCO_3$	Total acid
	mols.	mols.		mols.	mols.		mols.	mols.		mols.	mols.
4.75	–	0.00940	5.3	0.001	0.01400	7.23	0.005	0.00574	6.81	0.0025	0.00349
5.92	–	0.00270	7.3	0.001	0.00113	7.23	0.010	0.01148	6.76	0.0150	0.02150
6.12	–	0.00149	8.3	0.001	0.00101				6.81	0.0200	0.02780
6.58	–	0.00028									

TABLE III

EFFECT OF INCREASED [$KHCO_3$] AND [CO_2] AT pH 6.5 ON THE NITROGEN FRACTIONS OF POTATO DISCS AT 23° C. AND 72 HOURS*

Sample	[$KHCO_3$] per liter	Protein N per gram fresh weight	Soluble N per gram fresh weight	Protein N	Soluble N	Amino N	Amide N		Ammonia* N
							Stable	Unstable	
	equiv.	mg.	mg.	%	%	%	%	%	
Initial	–	0.58	1.44	28.7	71.3	56.5	8.4	7.4	
Final	0.005	0.90	1.16	43.7	56.3	45.6	4.9	5.8	
"	0.020	0.65	1.35	32.5	67.5	51.0	6.0	12.0	
"	0.040	0.65	1.40	31.7	68.3	50.8	6.3	10.7	

* Absolute units milligrams nitrogen per gram initial fresh weight. Results on a percentage basis are relative to total nitrogen. Negligible—0.001 mg. per gm. fresh weight.

SUMMARY OF THE EFFECTS OF THE COMPONENTS OF THE HCO_3^-/H_2CO_3 BUFFER SYSTEM ON BROMIDE UPTAKE.—The effect of bicarbonate and dissolved carbon dioxide on bromide uptake is complex. The components of the carbon dioxide buffer system *depress absorption.* Combined and free carbonic acid both depress bromide uptake and at pH 7.0 their relative effects are approximately equal. The indications are that the undissociated free acid in the system is less effective than the bicarbonate ion in retarding bromide uptake. A full interpretation of all the facts cannot evade effects which must be ascribed specifically to H and OH ions—effects which operate in such a fashion that reactions near neutrality become especially conducive to bromide uptake. This is particularly true when the total absorption is already reduced by the effects of added carbonic acid.

THE EFFECTS OF pH, DISSOLVED CARBON DIOXIDE, AND POTASSIUM BICARBONATE ON THE METABOLISM OF POTATO DISCS

THE EFFECT OF INCREASED $KHCO_3$ AND CO_2 CONCENTRATION AT pH 6.5.—The most striking feature of the effect of the components of the CO_2/HCO_3^- buffer system upon bromide uptake is that at a constant pH, bromide accumulation is progressively decreased, and eventually suppressed, by the simultaneous increase of potassium bicarbonate and dissolved carbon dioxide. This is true even though all other variables remain at values conducive to a high degree of salt accumulation. If bromide absorption is to be correlated with a particular aspect of metabolism, then this should be a process which is retarded by these treatments. Therefore, investigation of the metabolic processes so affected will go far to identify those which are essentially concerned in bromide absorption.

Discs exposed to aerated solutions of potassium bicarbonate at constant pH do not brown as much in the strong as they do in the dilute solutions or in distilled water. Usually (**21, 22**) increased concentrations of potassium salts tend to accentuate the surface browning reaction. Since bicarbonates are unusual in this respect, it is to be expected that the specific effects of the bicarbonate and dissolved carbon dioxide on metabolism would operate through processes which are linked with the activity of the oxidase (phenolase) system of the potato tuber. Experiments which were carried out at pH 6.5, show that this is the case; this is the pH at which the buffer effect is at its maximum.

From table III and figure 5, it is apparent that the protein synthesis which normally occurs in potato discs in distilled water or in dilute potassium bicarbonate solution is *almost completely suppressed* by an external concentration of 0.02 mols per liter and its attendant carbon dioxide concentration. This result, as well as much other evidence not given here (**21, 22, 23**) suggests that protein synthesis plays an indispensable part in the accumulation of bromide by potato discs.

Further analysis of the soluble nitrogen fractions shows that, as previously recorded (**21, 22**), the nitrogen used in synthesis is mainly amino-N.[4] The treatments which in the experiment of table III depressed synthesis (compare cultures at 0.005 and 0.020 equiv. $KHCO_3$) also retarded the utilization of amino-acid but *not to the same degree.* When there was little gain of protein in the tissue (cultures at 0.020 and 0.040 equiv. $KHCO_3$) more amino acid disappeared than reappeared as protein and the excess was accounted for by an increase in the unstable amide-like compound which has previously been noted (**21**) and which was then regarded as a possible reactive intermediary between amino-acid and protein. It thus appears that the combined effect of bicarbonate and carbon dioxide is not exerted exclusively upon the deamination of the amino-acids, but even more upon the

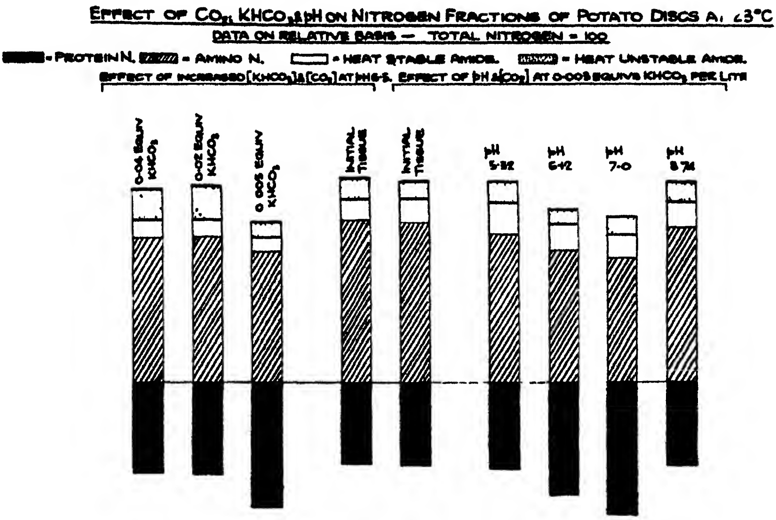

FIG. 5.

later stages of protein synthesis in which the reactive intermediaries are converted to protein. As the intermediates accumulate in bicarbonate cultures which depress synthesis they, in turn, tend to depress the deamination of the amino-acids. It will be recalled that when potassium salts stimulate synthesis they do so by affecting both the deamination of amino acid and the use of the reactive amides which are supposed to be intermediates in synthesis. When calcium salts depress synthesis, however, the unstable amides do not accumulate.

THE EFFECT OF pH AT CONSTANT BICARBONATE CONCENTRATION.—Superimposed upon the effect of bicarbonate and dissolved carbon dioxide, which at constant pH does not concern the H and OH ions, there is at any given

[4] Amino-N free from confusion with heat unstable amides which react both in the amide and the VAN SLYKE determination.

concentration of $KHCO_3$, an effect on protein synthesis which must be due primarily to these ions. This is shown by the data of table IV which are

TABLE IV

EFFECT OF PH IN $KHCO_3$ BUFFERED SOLUTIONS (0.003 EQUIV. PER LITER) ON THE NITROGEN FRACTIONS OF POTATO DISCS AT 23° C. AND 72 HOURS*

SAMPLE	PH	PROTEIN N PER GM. FRESH WT.	SOLUBLE N PER GM. FRESH WT.	PROTEIN N	SOLUBLE N	AMINO N	AMIDE N		AMMONIA† N
							STABLE	UNSTABLE	
		mg.	mg.	%	%	%	%	%	
Initial	.. ...	0.62	1.46	29.8	70.2	55.0	8.6	6.2	....
Final	5.32	0.63	1.42	30.7	69.3	51.7	11.2	6.3	....
"	6.12	0.82	1.25	39.6	60.4	46.3	9.2	4.9	....
"	7.00	0.95	1.09	46.6	53.4	43.6	8.4	6.4	....
"	8.74	0.59	1.39	29.8	70.2	54.0	9.0	6.5	.. ..

* Absolute units, milligrams nitrogen per gram initial fresh weight. Results expressed on a percentage basis are relative to total nitrogen.

† Negligible—0.001 mg. per gm. fresh weight.

illustrated in figure 6. It is clear that *protein synthesis is at a maximum at pH 7.0* and that *it declines both in more acid and more alkaline solutions.* It will appear later that similar results are obtained in phosphate buffered solutions although specific effects of phosphate and bicarbonate on protein synthesis are quite different. This effect of pH must, therefore, be due to H and OH ions specifically.

The detailed analyses of the soluble nitrogen fractions simply show that the effects of pH are exerted mainly upon the utilization of the amino-acid fraction. The changes in total soluble nitrogen and amino nitrogen run parallel throughout and they are complementary to the observed change in protein nitrogen (table V).

Combining now the results at constant pH and at constant bicarbonate concentration in figure 6, it is impossible to ignore the similarity between the effects of these treatments on protein synthesis and on bromide accumulation. In both cases the process in question is retarded and eventually suppressed by increased concentrations of bicarbonate and dissolved carbon dioxide; at a given salt concentration, it exhibits an optimum at a pH value of 7.0. It is to be concluded, therefore, that the specific effects of the components of the H_2CO_3/HCO_3^- buffer system and of pH on bromide absorption are linked with their effect on protein synthesis—a process with which bromide uptake in potato discs is closely associated.

EFFECT OF PH, BICARBONATE, AND DISSOLVED CARBON DIOXIDE ON THE RELATIVE ABSORPTION OF ANION AND CATION

The absorption of potassium in the experiments of table I presented a

TABLE V

EFFECT OF pH, $[KHCO_3]$ AND $[CO_2]$ ON ACCUMULATION OF POTASSIUM BY POTATO DISCS AT 23° C. DURING 70 HOURS

Exp. no.	[K] in external solution	$[KHCO_3]$ in external solution	Mean pH	Gain of K in sap	Exp. no.	[K] in external solution	$[KHCO_3]$ in external solution	Mean pH	Gain of K in sap	K absorbed in excess of Br
	equiv./l.	*equiv./l.*		*mg. equiv./l.*		*equiv./l.*	*equiv./l.*		*mg. equiv./l.*	*mg. equiv./l.*
1	0.001		4.75	− 9.6	2	0.011	0.001	7.30	11.0	
2	0.011		5.92	− 5.7	2	0.011	0.005	7.23	20.0	8.5
2	0.011		6.12	+ 0.2	2	0.011	0.010	7.23	14.5	7.7
2	0.011		6.58	+ 5.5	3	0.021	0.025	6.81	19.1	5.0
1	0.001		6.90	+17.4	3	0.021	0.015	6.76	32.9	30.0
3	0.021		7.70	+ 9.4	3	0.021	0.020	6.81	22.5	21.0

more difficult problem than that of bromide. It is complicated by the effect of the treatment on the high concentration of potassium which existed in the initial tissue (76 mg. equiv. per liter of sap) and which, under conditions unfavorable for absorption, may leave the cells. It is necessary to examine the results obtained to see whether these variables affect potassium and bromide absorption in a similar fashion and to ascertain whether the evident connection between protein synthesis and bromide uptake applies equally to potassium absorption.

The effect of pH on the absorption of potassium and on the potassium content of the initial tissue can be seen in the first part of table V.[5] The different experiments were conducted at various total potassium concentrations which are shown, though they were all at constant bromide concentration and in the absence of bicarbonate. It is clear that the concentration of the only mobile anion (Br), which was constant, had a greater control over the uptake of cations than the varying concentration of potassium sulphate in the range shown. If potassium sulphate concentration had any tendency to increase potassium absorption, allowance for this would merely accentuate the effect of pH to which reference will now be made.

At neutrality (strictly $pH = 6.9$) absorption of potassium and bromide was in equivalent amounts—a fact which has often been observed for potato discs in potassium bromide solutions. At more acid reactions, due to increased concentrations of carbon dioxide, there was an apparent decrease in the potassium absorbed. This was due to the effect on the absorption of potassium bromide but also to the effect of carbon dioxide on the loss of potassium which was present initially in the tissue. This loss was so great at acid reactions ($pH < 6.0$) that it entirely masked the potassium absorbed along with bromide. Although the data are limited at reactions more alkaline than pH 7.0, there is a strong suggestion that these also caused loss of the potassium previously stored in the cells. The obvious conclusion is that the tissue retains its stored potassium best under those conditions most suitable for protein synthesis.

Reference may be made here to a similar, though even more striking, case to which a similar conclusion applies. After prolonged storage of tubers at low temperatures, potato discs no longer retain their salts against aerated distilled water (**17**, p. 536); they lose the capacity to grow as shown by meristem formation in moist air, and *it is now known that they also lose their ability to synthesize protein.*[6] Though as yet unpublished, these results strengthen the conviction that the observed effect of pH and carbon dioxide concentration on the loss of potassium from the cells is causally connected with their effect on protein synthesis (fig. 6). This suggests that,

[5] For these potassium analyses we have to thank members of the laboratory of Plant Nutrition, University of California, Berkeley, California.

[6] Data in thesis of T. K. Ramamurti, University of London.

in order to retain their solutes against distilled water, the cells cannot merely remain static; they must also be capable of synthesizing protein. The evident connection between protein synthesis and the ability of cells to retain potassium in their sap, recalls previous observations that the discs must maintain an unexpectedly high rate of respiration in order to retain

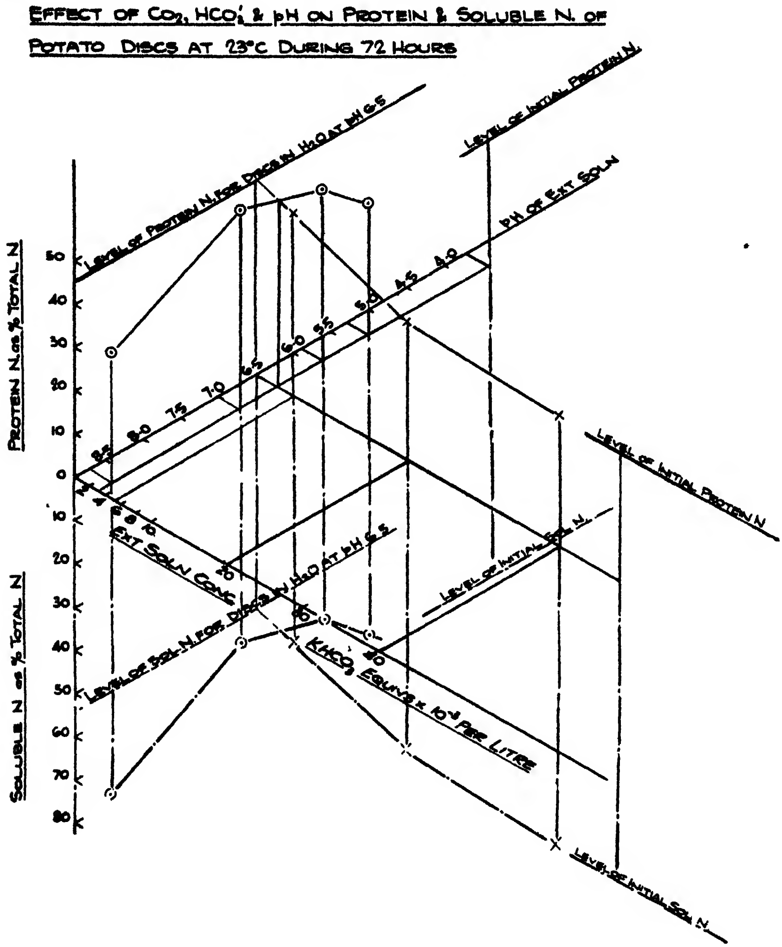

FIG. 6.

their salts (**16**, pp. 215–234). These results stand in contrast to the long-cherished conception of a passive "semi-permeable" structure by which cells retain the solutes contained in their vacuoles. It must be recognized that the metabolic processes of respiration and protein synthesis are equally necessary to retain salts *after they are accumulated* as they are to produce the high internal salt concentration *de novo*.

The effect of bicarbonate and dissolved carbon dioxide concentration at constant pH is revealed by two series of data from experiments at different total potassium concentrations. In the presence of added bicarbonate, the absorption of potassium always greatly exceeded the absorption of bromide, even at reactions close to neutrality (pH 7.23 and 6.8). At contant pH, the potassium absorbed in excess of bromide increased with bicarbonate concentration up to an optimum concentration above which, presumably owing to the effects of dissolved carbon dioxide, further increase reduced the total absorption of potassium as well as its excess over the bromide absorbed. There can be little doubt that the *potassium absorbed which was unaccompanied by bromide was absorbed along with bicarbonate ion* and that this process occurred under conditions such that *protein synthesis and bromide uptake were reduced almost to zero* (culture at 0.020 mol. $KHCO_3$ and pH 6.8, table V). The absorbed bicarbonate, however, did not remain as such in the sap; when this was acidified, it yielded little more carbon dioxide than did the normal sap. The presumption is that potassium bicarbonate was absorbed as such and reacted with the organic acids of the sap. This process represents at least a transitory means by *which the cells accumulate potassium independently of protein synthesis and of growth* although it can persist only so long as the stored reserves of organic acids remain or are replenished. The final result would be the same if potassium exchanged for hydrogen ion supplied by the organic acid. Some may prefer this interpretation but it leaves the rôle of bicarbonate in the solution without explanation.

Further work on this point is necessary but there seem to be grounds for homologizing this uptake of potassium by potato discs with the brief but rapid uptake of potassium by "low salt" barley roots during which, if it occurs from potassium bicarbonate, organic acids increase in the cell sap. Potassium may be absorbed, therefore, by distinct mechanisms which bear different relationships to metabolism. The absorption of potassium from bicarbonate solutions presents a very special case—it is clearly less dependent upon certain aspects of metabolism (protein synthesis and processes linked with it) than the absorption of potassium bromide. The extent to which it is conditioned by oxygen in the external solution remains an interesting problem for the future.[7]

The unequal absorption of anion and cation may be a contributory cause of the metabolic effects which obtain in bicarbonate solutions. HOAGLAND's observation that barley roots respond to bicarbonate absorption by actual increase in their organic acid content has been noted. Indications that the centers of protein synthesis in potato discs respond to unequal absorption

[7] In barley root experiments, HOAGLAND and BROYER have shown that the absorption of potassium from bicarbonate solutions does not occur at very low oxygen concentrations (private communication to the authors).

of cation and anion as though they operate best under conditions of strict neutrality, have been noted elsewhere (**21**). Equal uptake of both ions from some potassium salts (KBr) with its concomitant synthesis and the fact that both synthesis and uptake are increased by greater concentrations, contrast with the unequal uptake of calcium salts (accentuated perhaps by the possible fixation of the cation as insoluble compounds) and the depression of synthesis which greater concentrations cause. In potassium bicarbonate solutions the final effect is as if the cation were absorbed unaccompanied by anion (or exchanged for hydrion) and this condition of ionic unbalance is associated with a markedly depressed protein synthesis. Moreover, both in phosphate and bicarbonate buffers at constant total acid the synthesis is a maximum at a neutral external reaction of 7.0. Hence the relation between protein synthesis and salt uptake is not merely that synthesis is a vital property of growing cells which makes uptake possible. It is that continued absorption, like synthesis, does not tolerate the consequences of ionic unbalance due to unequal intake of anions and cations by the cells and it is favored by external solutions with reactions close to strict neutrality. If disturbance in the metabolic machinery is the consequence of such ionic inequality, one may well look for its explanation in the behavior of the organic and amino acids. The whole tendency is to conserve the latter in the presence of bicarbonate and avert their conversion to protein.

The effect of pH, potassium bicarbonate, and carbon dioxide concentration on the respiration and metabolism of carbohydrate of potato discs

The direct determination, by carbon dioxide evolved, of the respiration of potato discs in solutions rich in bicarbonate and dissolved carbon dioxide presented too great technical difficulties to be profitable. Indirect determinations by the change in total carbon in the tissue due to respiration was complicated by the loss of carbon which the tissue sustains when it is blotted dry with paper (**23**) and by the absorption of bicarbonate from the external solution. Consequently, the effect of the treatments already described on respiration cannot be stated very precisely. It is clear, however, that respiration, like the phenolase activity and protein synthesis, was retarded at pH 7.0 in the presence of bicarbonate (0.020 N) and dissolved carbon dioxide (solution in equilibrium with 20 per cent. CO_2 by volume). This was demonstrated by using tissue of a variety different from that used in experiments 1, 2, and 3, and by comparing the total heat content (bomb calorimeter determination), starch and sugar content of initial washed discs, and comparable samples after 70 hours of contact with either bicarbonate solution at pH 6.5 or the equivalent strength of potassium sulphate.

In the tissue in the bicarbonate solution the hydrolysis of starch to sugar (final sugar, 0.24 gm.; final starch, 3.10 gm. per 40 gm. initial fresh weight) was retarded in comparison with that which received the sulphate treatment (final sugar, 0.34 gm.; final starch, 2.75 gm. per 40 gm. initial fresh weight). The total carbohydrate recovered (starch + starch equivalent of final sugar) was greater in the bicarbonate culture than in the sulphate. The still outstanding loss of carbohydrate (initial tissue contained 4.16 gm. and the bicarbonate treated 3.32 gm. total starch per 40 gm. fresh wt.) in the tissue treated with bicarbonate solution at pH 6.5 was due partly to carbon losses incurred in blotting the discs (**23**), and partly to respiration or other metabolic processes which were not separately measured. One must make the same allowance (estimated at 0.42 gm. per 40 gm. initial tissue) for loss of carbohydrate owing to the formation of the surface film of mucilage in the bicarbonate series, as that which is necessary to "balance" the carbohydrate balance sheet of the potassium sulphate series. It then appears that at pH 7.0 in contact with 0.020 N potassium bicarbonate and the appropriate carbon dioxide concentration, 40 gm. of this tissue (in which protein synthesis was depressed but not completely eliminated) respired 0.42 gm. of starch in 70 hours as against 0.64 gm. in bicarbonate and carbon dioxide-free solutions. The total heat change in the discs also indicated that respiration was depressed in presence of bicarbonate and dissolved carbon dioxide but the precise effect must be determined by other methods. Knowing the general parallelism between the effects of other salts on respiration and protein synthesis (**23**) and also the phosphate experiments in this paper, the trend of the effect of the bicarbonate and carbon dioxide treatments on respiration may be inferred from their effects on protein synthesis which are here recorded. Adopting this standpoint, the now oft-repeated parallelism between bromide absorption and respiration of potato discs would again emerge—a treatment (increased bicarbonate concentration) which depresses bromide uptake has a similar effect on respiration.[8]

Carbon dioxide has somewhat unexpected results on living cells. The observations of THORNTON (**25**) and FIFE and FRAMPTON (**3**) have given prominence to an effect of carbon dioxide on the pH of the cell and show that this is not always predictable on *a priori* grounds. FIFE and FRAMPTON showed that the explanation of the unexpected shift toward alkalinity in the sap of carbon dioxide-treated cells lies in its effect on systems in the cells which catalyze the hydrolysis and re-synthesis of amides—reactions which occur only in the living tissue in the presence of oxygen (**3, 25**). Actual tests showed that this effect did not enter appreciably into the behavior of

[8] Evidence from the researches of A. ULRICH on barley roots (in course of publication) suggest that the respiratory quotient may be altered when accumulation of K occurs from a solution of $KHCO_3$. Further investigation of this possibility with reference to potato tissue would be of interest.

immersed potato discs at pH 7.0. FIFE, at the author's request, made tests in 1934 which showed that the shift in pH toward alkalinity in the sap expressed from potato discs which have been exposed to high concentrations of dissolved carbon dioxide (equilibrium with 2 atmospheres pressure CO_2) was only slight (pH 0.26). At the same time, the detailed analysis in tables III and IV did not show the free ammonia to be expected if amide hydrolysis had occurred extensively. It will be recalled, however, that the tissue at pH 7.0 in solutions rich in bicarbonate and dissolved carbon dioxide did not show the usual symptoms of oxidase activity and it is here, through some esential property of the living protoplast, that the mechanism must be sought. From the evidence presented it is clear that the effect of bicarbonate and dissolved carbon dioxide on metabolism embraces the nitrogen compounds although mainly in a way other than that which FIFE and FRAMPTON (3) described for the beet plant exposed to carbon dioxide gas. The response of beet leaves to CO_2 is rapid and considerable (increases of pH approaching 1.0 pH unit were observed by FIFE and FRAMPTON in 60 minutes). THORNTON'S work was done on much larger masses of tissue in a gas phase and the response, though considerable, required a longer period. A possibility that thin discs would react even more than the large masses was evidently not realized. This difference is most probably due to factors incidental to the use of discs immersed in aerated solution, though the possibility that the reversibility of the effect, which FIFE and FRAMPTON observed for beets, is so accentuated in these discs that their reaction is reversed before sap can be expressed and measured may have to be considered. Even if this were the case, the clue to the metabolic effect of carbonic acid and bicarbonate solutions should be with the permanent, rather than the transient, results of such treatments.

Experiments made on the effect of pH and phosphate concentration on the respiration and nitrogen metabolism of potato discs afford an interesting comparison with the bicarbonate series from which the effects specifically due to H and OH ions may be inferred.

THE EFFECT OF pH AND PHOSPHATE CONCENTRATION ON THE METABOLISM OF POTATO DISCS

The trend of the effects of total phosphate concentration and of pH in phosphate buffered solutions is shown by the results of two series of experiments. One is conducted at constant pH and the other at constant total concentration of total phosphate and potassium; otherwise both are conducted under identical conditions of time, temperature, aeration, etc. These variables are fixed at the same arbitrary values used in the bicarbonate experiments so that direct comparisons can be made between the two series of experiments.

The effects due to varying concentration of total phosphate were determined at pH 6.9. This is a reaction close to strict neutrality and at the pKa value of phosphoric as a dibasic acid where the buffering effect of potassium dihydrogen phosphate in presence of potassium monohydrogen phosphate is at its maximum. The effects due to pH were determined at constant concentration of 0.020 mol per liter of total phosphate. To eliminate confusion due to variable concentration of potassium, the solutions had their potassium content raised where necessary, to a constant level of 0.040 equivalent per liter, by the addition of potassium since the sulphate ion has only slight influence on the metabolism of potato discs. The composition of the solutions used is given in table VI.

TABLE VI

COMPOSITION OF BUFFER MIXTURES FOR EXPERIMENTS AT CONSTANT PHOSPHATE AND POTASSIUM CONCENTRATION*

pH	Solution A to 2 liters	Solution B to 2 liters	Solution C to 2 liters
	ml.	*ml.*	*ml.*
8.04	133	137.0	
7.46	133	112.0	25.0
6.95	133	73.0	64.0
6.48	133	33.0	104.0
5.96	133	14.6	122.4
6.82			270.0

* Stock Soln. A = 0.3 M KH_2PO_4.
Stock Soln. B = 0.3 M KOH.
Stock Soln. C = 0.15 M K_2SO_4.

To arrive at these desired mixtures stock solutions of 0.3 M KH_2PO_4 and KOH were prepared and, using the glass electrode, the titration curve of the one against the other was determined and from this the volume mixtures necessary for any desired pH could be ascertained.[9]

For the experiment at constant pH a phosphate buffer mixture at pH 6.9[10] was prepared which was then diluted so that the desired total phosphate concentrations were obtained; namely, 0.002 M, 0.01 M, 0.025 M, and 0.040 M. The last was too strong and, because of the less turgid condition of the tissue in this solution, no safe conclusions could be drawn from this culture. These data are therefore omitted.

The effect of the above treatments can be represented by a method similar to that previously adopted for the bicarbonate experiments. Respiration rate and protein, or soluble nitrogen, content of the discs were plotted on isometric paper against *both* pH of the external solution *and* the

[9] See HOLT, LAMER, and CHOWN (7) for a discussion of the ionic equilibria in phosphate solutions at different pH's; also CLARK (2).

[10] One liter of 2 M/5 KH_2PO_4 + 720 ml. 2 M/5 KOH, the mixture diluted to 2 liters.

total phosphate concentration. The data obtained identify two sections cut through a solid model, the surface of which describes the inter-relationships of the three variables concerned. Sufficient data to identify such surfaces completely would involve a rather formidable investigation which it might be difficult to complete before the changes which ensue during storage affected the behavior of the tissue. The experiments were so planned, however, that the more limited data available permit one to discern the general trend in the effects which were obtained.

RESPIRATION.—The time curve of respiration of tissue in phosphate buffered solutions at pH 6.9 is identical in form to that already shown for tissue in distilled water or certain neutral salt solutions ($CaCl_2$). It consists of an initial period during which the respiration rises to a value which is subsequently maintained for long periods. Phosphate cultures at acid or alkaline reactions introduced further complications into the initial period and it is not proposed to deal with these at length here. It will suffice to state that the carbon dioxide removed by the air stream from the cultures in the first period increased progressively from the more alkaline to the more acid solution. This was doubtless due to the bicarbonate which remained in the more alkaline solution. Later in the time drift cultures, both more acid and more alkaline than pH 6.9, it produced a transient increase in respiration; this had, however, elapsed before the period of 26 to 72 hours to which particular attention will be directed.

The effect of increased concentration of total potassium phosphate on respiration is revealed by the series at constant pH (table VII). Those

TABLE VII

EFFECT OF EXTERNAL PH ON RESPIRATION* OF POTATO DISCS IN POTASSIUM PHOSPHATE BUFFERS (0.020 M) OF CONSTANT K CONTENT (0.040 EQUIV.) AT 23° C.

External pH	5.96	6.48	6.95	7.46	8.04
Respiration rate	0.207	0.299	0.325	0.302	0.234
Relative respiration rate	63.8	92.0	100.0	93.0	72.0

* Respiration rates = mean rates in mg. CO_2 per gm. initial fresh wt. per hour for period 26 to 72 hours of exp.

Relative respiration rate = culture at pH 6.95 as 100.

effects are due either to potassium or to the simultaneous and proportional increase in $H_2PO_4^-$ and HPO_4^{--}; the relative amounts of these are equal in solutions of pH equal to the pKa value of the second dissociation of phosphoric acid (pH = 6.9). Total phosphate concentration increased respiration at pH = 6.9 (table VIII, fig. 7); this might be expected from the prevalent belief that hexose phosphate plays a prominent rôle in respiration.

It is well to emphasize, however, that the increase observed is not conspicuously greater than the response obtainable with potassium nitrate and

TABLE VIII

EFFECT OF TOTAL CONCENTRATION OF POTASSIUM PHOSPHATE BUFFER ON RESPIRATION RATE* OF POTATO DISCS AT pH 6.9 AND 23° C.

Molar concentration phosphate	0.0	0.002	0.010	0.025
Respiration rate	0.193	0.204	0.245	0.367
Relative respiration rate†	100.0	106.0	127.0	190.0

* Respiration rates = mean rates in mg. CO_2 per gm. initial fresh wt. per hour for period 26 to 72 hours of exp.
† Relative respiration rate ÷ culture in distilled water = 100.

it is actually less than the response which has been obtained in ammonium sulphate and nitrate solutions (unpublished experiments). The interpretation of the mechanism of the phosphate response can be deferred, but it is clear that the doubtful specific effect of phosphate, unlike that of bicarbonate, is to increase respiration.

Despite the contrast in the effects due to the two anions, the effects of pH on respiration in phosphate buffered solutions (table VII, fig. 7) are similar to those of pH on protein synthesis (and presumably also on respiration) in bicarbonate buffered solution. After the respiration had attained somewhat steady levels during the last 36 hours of the treatments, the maximum respiration occurred at pH 6.9. When data from the two experiments are combined (fig. 7) the probable form of the surface which depicts the effect of phosphate concentration and pH on the respiration of potato discs can be visualized.

THE GENERAL EFFECT OF pH ON RESPIRATION.—The literature contains no other record of the effect of phosphate concentration and pH on the respiration of potato discs similar to figure 7. Reported attempts to show the effect of pH suffer from inadequate appreciation of the variables which affect the behavior of potato discs. LEMMON (8) and BOSWELL (1), both working with potato tissue, used the WARBURG manometric method—a technique the full implications of which have not been adequately investigated relative to the behavior of potato discs. LEMMON was primarily concerned with the effects of pH on respiration but used buffer solutions of such varied composition that the effect of pH *per se* could not be segregated from that due to other variable components of the solutions. This was doubtless responsible for the erratic behavior observed.

BOSWELL though not primarily concerned with the effects of either salts or pH on respiration, used such short time periods (0 to 3 hours) that it is questionable if the tissue had fully responded either to the salt treatment or to the oxygen content of the solution if this was indeed in equilibrium with air. BOSWELL did not state the full composition of his buffer solutions; *e.g.*, the nature and concentration of the cation. He remarks that his results

show that for the pH range of 5.29 to 8.04, the oxygen uptake is unaffected between pH 5.59 and 6.81 "falls away slightly below 5.9 and rises above 6.81." No final conclusion can be drawn from his figures which merely show that in his experiments the effect of pH, if indeed pH was the causal factor, was erratic. The pH effects described in this paper, whatever their ultimate explanation, are clearly consistent with the other results of this

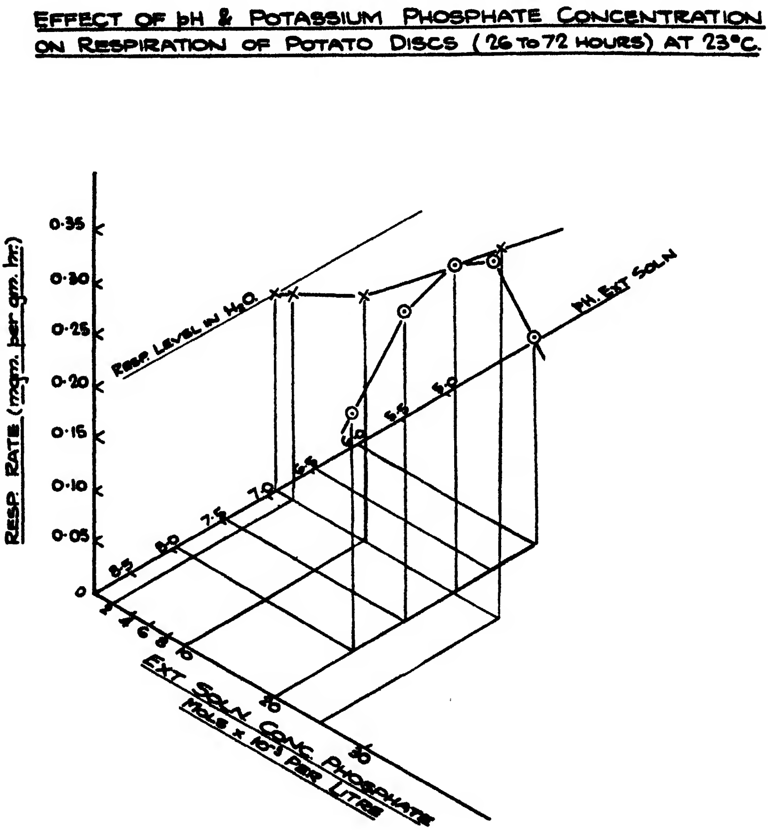

FIG. 7.

series of investigations and they form part of a comprehensive, general picture of the behavior of potato tissue.

With respect to other tissues there are inconsistencies in the relation of respiration to pH. To cite but two cases: LYON's (**10**) results with Elodea showed greater respiration with increasing alkalinity beyond pH 7.0; whereas THOMAS (**24**) claims that respiration increases with acidity in carrots.

Even the effect of phosphate concentration on respiration is not free from contradictions. LYON (**9**) using Elodea treated with phosphate solu-

tions at a "neutral reaction" for one hour—treatment which if prolonged to 30 hours caused death—showed that increased phosphate concentration stimulated respiration although preceded by a very brief depression. He ascribed this effect to the PO_4 ion and related the effect to the "p PO_4" (**11**). BOSWELL's data on the potato are different but difficult to interpret. The cation content of the buffer solutions was not specified, and during 3 hours the effect of increased phosphate concentration at pH 5.5 was to decrease respiration. Whenever a preliminary decrease in respiration of potato discs, due to phosphates of sodium or potassium, has been observed in our investigation it has always been succeeded by a prolonged period in which phosphate increased respiration. The general conclusion that phosphates decrease the respiration of potato discs should not be drawn from BOSWELL's data. LYON (**11**) sees in his well-known observation that inorganic phosphate will stimulate the activity of potato oxidase preparations (aqueous extracts of pulp) so that—*in presence of phosphates only*—they can oxidize sugar, evidence that the mechanism of the phosphate effect on aerobic respiration is a catalytic effect on the oxidases. Recognizing that LYON's tissue pulp extracts may have also contained nitrogen compounds, and broadening the usual range of oxidase substrates to include their secondary effects on amino-acids, such an explanation would be consistent with the standpoint of this paper.

NITROGEN METABOLISM.—Using the initial and final tissue from the experiments of tables VII and VIII, the changes which occurred in the protein nitrogen, soluble nitrogen, and the various components of the soluble nitrogen were determined by the methods which have been described The data in absolute units are to be found in tables IX and X. The recov-

TABLE IX

EFFECT OF CONCENTRATION OF POTASSIUM PHOSPHATE BUFFER SOLUTION AT PH 6.9 ON THE NITROGEN FRACTIONS OF POTATO DISCS AT 23° C. DURING 72 HOURS*

SAMPLE	CONCENTRATION POTASSIUM PHOSPHATE IN EXTERNAL SOLUTION	TOTAL N PER GRAM	PROTEIN N PER GRAM	PROTEIN N	SOLUBLE N	AMINO N	PERCENTAGE AMIDE N		AMMONIA† N
							STABLE	UNSTABLE	
	M	*mg.*	*mg.*	%	%	%	%	%	
Initial tissue		2.02	0.59	29.2	70.8	58.4	9.4	6.4	
Final	0.0	2.02	0.90	44.6	55.4	44.6	5.4	7.4	
"	0.002	2.09	0.93	44.6	55.4	46.7	6.2	1.0	
"	0.010	2.05	0.99	48.3	51.7	42.9	6.8	0.5	
"	0.025	2.03	1.20	59.2	40.8	34.5	6.4	0.5	
"	0.040	2.03	1.08	53.2	46.8	38.4	7.4	0.2	

* Absolute units = mg. N per gm. initial fresh weight. Results on percentage basis are relative to total nitrogen.

† Negligible, order of 0.001 mg. per gm. fresh wt. No effect of conc.

TABLE X

EFFECT OF PH IN POTASSIUM PHOSPHATE BUFFERED SOLUTIONS (0.020 M PHOSPHATE) ON THE NITROGEN FRACTIONS OF POTATO DISCS AT 23° C. DURING 72 HOURS*

SAMPLE	pH	TOTAL N PER GRAM	PROTEIN N PER GRAM	PROTEIN N	SOLUBLE N	AMINO N	AMIDE N		AMMONIA† N
							STABLE	UNSTABLE	
		mg.	mg.	%	%	%	%	%	
Initial tissue		2.02	0.59	29.2	70.8	57.4	8.9	6.44	
Final	5.96	2.02	0.68	33.6	66.4	53.5	8.4	5.45	
"	6.48	2.00	0.83	41.5	58.5	50.0	6.5	0.55	
"	6.95	2.03	1.14	56.2	43.8	36.9	6.4	0.59	
"	7.46	2.00	0.78	39.0	61.0	54.0	8.0	0.50	
"	8.04	2.01	0.60	29.9	70.1	51.8	8.0	4.98	

* Absolute units = mg. N per gm. initial fresh weight. Results on a percentage basis are relative to total nitrogen.

† Negligible, order of 0.001 mg. per gm. fresh wt. No effect of pH.

ery of the initial total nitrogen of the tissue was quantitative and the changes which occurred are shown relative to the total nitrogen in figure 8. In this figure, protein nitrogen is represented by histograms below the line and the absolute nitrogen fractions by histograms above the line. In analyzing tissue from phosphate cultures it is essential to use the trichloracetic acid method for the determination of protein nitrogen. The alcohol method gives anomalous results since some protein is soluble in hot alcohol in the phosphate treated cultures.

The outstanding fact is that the salt or pH treatment caused respiration wherever it induced greater protein synthesis. This is shown clearly by a comparison of the histograms which represent the respiration rate (relative to standard treatments to which the value 100 is assigned) and those which represent the protein nitrogen content of the tissue (fig. 8). This is yet another, though very striking, example of the parallelism which exists between protein synthesis and respiration in potato discs. It has more than usual interest since it suggests that *even in its response to phosphate, the respiration of potato discs is modified through that same respiratory component which is linked with nitrogen metabolism.* This component is not controlled by sugar concentration, and is responsible for those other effects of inorganic salts on the respiration of potato discs that have been described in earlier papers.

The full implications of an investigation by RICHARDS (**14**) cannot be discussed here. It should be noted even though it treats of a problem apparently somewhat remote from the present one, since it correlates phosphorus deficiency during the growth of barley seedlings with low protein content and low respiration.

An explanation of the phosphate response without recourse to the hexose

phosphates and their reactions may occasion some surprise. At this stage finality is clearly impossible but the implication is that even potassium phosphates act upon metabolic processes which, as yet, appear to be somewhat remote from the metabolism of glucose via hexose phosphates. It is true that out of the increasing knowledge of the specific enzymes which

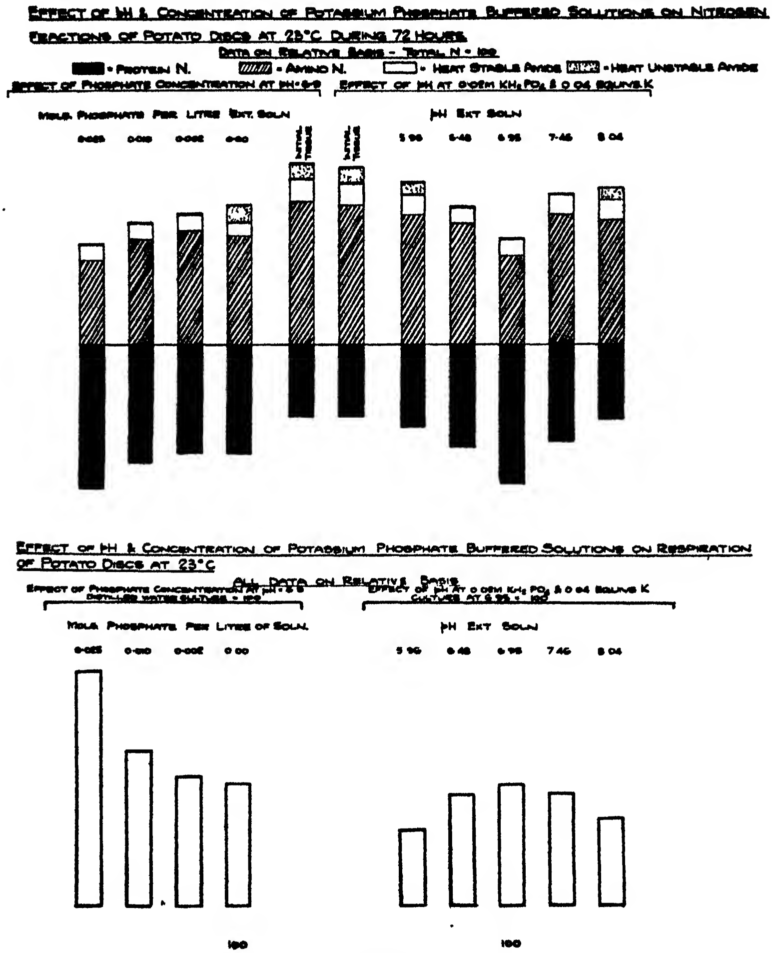

FIG. 8.

catalyze the reactions of the hexose phosphates an explanation of the effect of pH on respiration may be forthcoming. A notable achievement is that of HANES (4, 5) who has isolated a phosphorylase from crude potato sap which will reversibly convert starch to glucose-l-phosphate. This enzyme has a pH optimum at 6.4, but the enzyme machinery (phosphoglucose conversion system of HANES) which converts this substance to hexosediphos-

phate and thence catalyses the splitting of the carbon chain, has apparently a pH optimum at a reaction more alkaline than pH 7.0. It is, therefore, not inconceivable that processes dependent upon the consecutive action of such enzymes might appear to be favored by a reaction of 7.0. The possible connection of the phosphorylation of sugar with deamination of amino acids and protein synthesis is remote. It still seems, however, that phosphates and pH must exert some direct effect upon nitrogen metabolism in potato discs apart from the effects exerted upon the main line of carbohydrate breakdown by the route which the latter is commonly believed to take.

In the presence of phosphate, as of other salts, the bulk of the nitrogen used in protein synthesis was drawn from the amino-nitrogen fraction; it will be seen from figure 8 that the changes in amino nitrogen are closely parallel to the increase in protein nitrogen. An outstanding feature is, however, the effect of phosphate upon that part of the total soluble nitrogen which has been designated "heat unstable amide." In the initial tissue this fraction usually comprises about one-third of the soluble nitrogen other than the true amino nitrogen. The stable amide usually decreases, but the unstable amide fraction usually increases when the tissue metabolizes in aerated distilled water (table IX, fig. 8). In all phosphate solutions with reactions between pH 6.5 and 7.5 the content of "unstable amide" in the tissue was reduced to a low level.

Comparing the tissue which was subjected to distilled water and to 0.025 M phosphate (table IX, fig. 8), it is clear that of the nitrogen for the *extra* synthesis of protein which was stimulated by the salt, approximately 70 per cent. was derived ultimately from *amino nitrogen*. The remainder, however, is accounted for by the utilization of the unstable amide fraction; this was so stimulated by phosphate that, although normally increasing in the aerated discs, it was used more rapidly than it was produced and thus the amount present in the tissue decreased. It will be recalled that the unstable amide fraction (**21, 22**) has previously been regarded as a possible reactive intermediary between amino acid and protein and this further evidence that the reserves of this substance are depleted by yet another salt treatment which stimulates synthesis and respiration is suggestive in this connection. Conversely, at those reactions (pH 6.0 to 8.0), protein synthesis and respiration were both depressed, and the stimulating effects of phosphate were no longer evident because the inherent capacity of the tissue tended to maintain its initial store of unstable amide even in contact with phosphate.

The effects of pH on protein synthesis in phosphate and bicarbonate buffered solutions can be compared by reference to figures 6 and 9. The contrast in the specific effects due to phosphate and bicarbonate concentration at constant pH is evident; and also the fact that, *under each buffer*

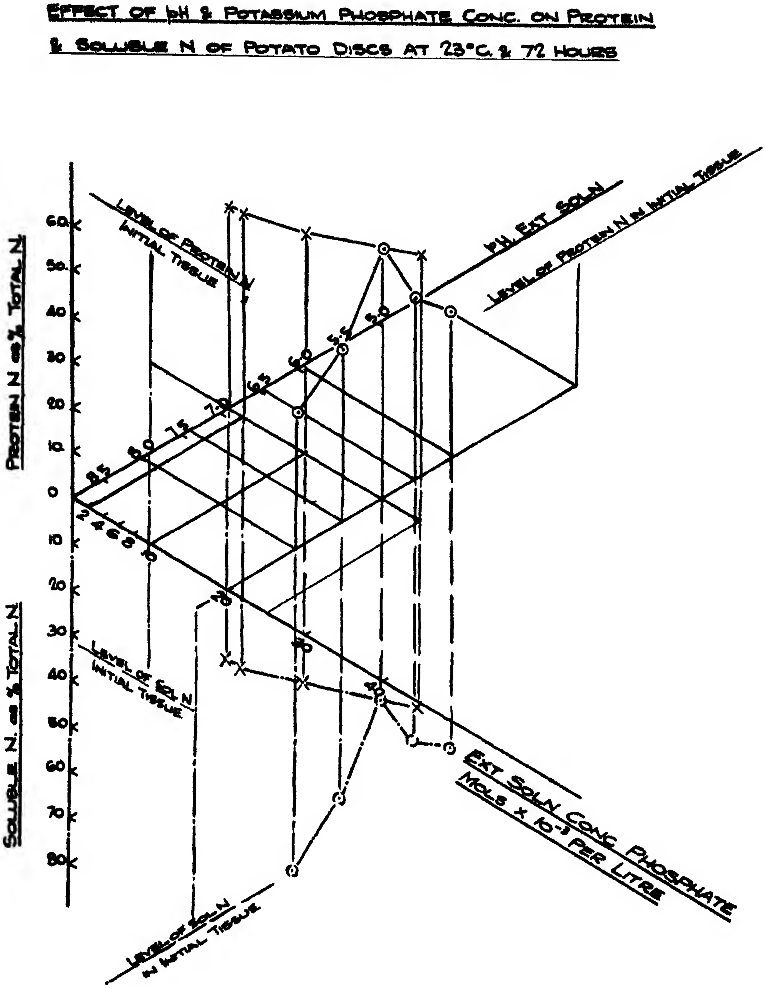

FIG. 9.

system, when the salt and other treatments were conducive to active protein synthesis, this reached a maximum at pH 7.0 and decreased to zero at more acid and more alkaline reactions. This effect can be attributed only to the direct effect of H^+ and OH^- upon the mechanism of protein synthesis. The obvious similarity between figure 7, which depicts the effect of pH and phosphate concentration on respiration, and figure 9 which shows the effect of similar treatments on protein synthesis, needs no further comment.

Figures 6 and 9 are reminiscent of the effect of pH upon the enzymatic synthesis of protein in concentrated protein hydrolysates. It was shown by WASTENAYS and BORSOOK (26) that the synthesis reached a maximum at a

pH of 4.0 in the case of egg albumin and the curve of synthesis as affected by pH was symmetrical about this reaction. They considered the possibility that this was due to the dissociation of an amphoteric component of the system although no component had an appropriate iso-electric point. The protein synthesis pH curves for potato discs are of a similar type. It is unlikely that the isoelectric properties of any amino acid present in potato determines the pH optimum at 7.0 and this appears to be attributable to some effect of the properties of water on the synthesis.

Salient features of the effect of pH, bicarbonate, dissolved carbon dioxide, and phosphates on the behavior of potato tissue

At pH 6.9, increased concentration of potassium phosphate increased both the respiration rate and protein synthesis of potato discs. In this respect phosphates behaved in a manner similar to potassium salts, in their reactions with other anions (Cl, Br, NO_3). It is thus clear that the stimulating agent is the potassium ion although its effect may be modified by the accompanying anion. Despite the prevalent idea that phosphates play an essential rôle in respiration, the stimulating effect of phosphates on the respiration of potato discs was not greater than that due to equivalent concentrations of other potassium salts. There is strong reason for the belief that the extra respiration stimulated by potassium phosphate was primarily due to that same component of respiration which is stimulated by the potassium ion as affected by other potassium salts.

At constant potassium and phosphate concentration both respiration and protein synthesis were at a maximum at an external reaction close to strict neutrality; at reactions somewhat more alkaline than pH 8.0 and more acid than pH 5.9 protein synthesis vanished. It is noteworthy that in potato discs the maximum protein synthesis does not occur in solutions at the same external reaction as that at which the extracted tissue proteins are iso-electric, *e.g.*, pH 4.4. [See Pearsall and Ewing (**13**) as suggested by Pearsall and Priestley (**12**) on theoretical grounds only.]

Potassium bicarbonate solutions in the presence of free carbonic acid and at pH 7.0 have a quite different effect upon metabolism since they depress protein synthesis and oxidase activity progressively as the salt concentration is increased. This is the *only case yet encountered in these investigations in which an increased concentration of a potassium salt decreased the respiration and metabolism of potato discs. It is evident that this effect is due specifically to HCO_3^- and H_2CO_3.* Although the available data are limited, it is clear that the metabolism of carbohydrate and respiration were also depressed by these salt treatments; the trend of the effect of bicarbonates and pH upon these processes should be similar to that found, and more fully investigated, for protein synthesis.

Increased concentrations of potassium bicarbonate and free acid at constant pH depress, and eventually suppress altogether, *both protein synthesis and also the accumulation of the bromide ion.* These facts again imply that the processes of *respiration, protein synthesis, and bromide uptake in potato cells are all closely linked together.* The data also show, however, that potassium was absorbed even from bicarbonate solutions which suppressed protein synthesis and bromide uptake. The presumption is that this was accompanied into the tissue by bicarbonate, which then reacted with the organic acids of the tissue since the bicarbonate concentration of the sap did not increase. Such absorption of potassium seems not to be as essentially related to growth as the simultaneous uptake of potassium and bromide. Treatment with solutions of bicarbonate and free carbonic acid affects the potassium previously stored in the potato cells in a manner which suggests that they *retain their salts most effectively under conditions which are optimum for protein synthesis.* All the evidence, therefore, reinforces the view that protein metabolism plays an essential rôle in the production and maintenance of ionic accumulation gradients.

The depressing effect of bicarbonates upon metabolism and bromide uptake cannot be ascribed wholly to the combined carbonic acid or to the free acid but both play a part. It seems that the bicarbonate ion present is more potent than the undissociated free acid. Three-dimensional models portray the inter-related effects of pH, bromide absorbed in the sap, protein synthesis, and potassium bicarbonate (total carbonic acid concentration in the solution). These show that the influence of added bicarbonate and free carbonic acid on bromide uptake or protein synthesis reaches a maximum at pH 7.0. Similarly, protein synthesis is, at any given salt ($KHCO_3$) concentration, at a maximum at pH 7.0 and declines to zero at reactions as alkaline as pH 8.7 and as acid as pH 5.3.

The almost identical effect on protein synthesis of solutions of different pH but constant salt concentration which were obtained with the different buffer systems employed, although the constituent salts (bicarbonate and phosphate) have distinct and opposed effects on metabolism, is evidence that the effect of pH at constant potassium concentration which is in question *is mainly due to the H and OH ions* and not to other ions or molecules in the buffer systems concerned. The importance of the reaction pH 7.0, at which protein synthesis is at a maximum and the depressing effect of added bicarbonate a minimum, is an indication that the process is limited in some way by the properties of water; the physico-chemical properties of plant amino-acids and proteins (iso-electric points) do not show maxima or minima at a pH of 7.0.

The effects of both phosphate and bicarbonate upon the nitrogen metabolism of the discs ultimately become apparent upon the utilization of the

amino-acid reserves. In both cases, the color reactions which the treatments induce, suggest that the activity of the phenolase in the tissue is a potent part of the mechanism of the salt effects. In both cases, however, there is evidence which suggests that the effect of the salts extends beyond the possible rôle of the phenolase in the deamination of amino compounds. The unstable, amide-like substance, to which previous reference has been made, is so affected by phosphate and bicarbonate treatments that a relative increase in protein synthesis caused by the salt results in a relative decrease of unstable amide and vice versa. On the highly probable assumption that the substance in question is formed from sugar and the products of deamination of amino acids and is an intermediate in protein synthesis, it becomes clear that *the effects of phosphate and bicarbonate extend to the later stages of the conversion of such intermediates to protein and are not confined to the deamination mechanism alone.* Of course, phenolase may also be involved in these stages.

Despite the fact that metabolic processes (respiration and protein synthesis), which are clearly connected with the ability of the tissue to absorb salts, are favored by a neutral reaction of pH 7.0 there is, nevertheless, a wide range of pH (6 to 8) within which the bromide uptake is not conspicuously affected by pH.

The investigation emphasizes again the ramifications of the biochemical effects due to ions in the external solution which extend to all phases of the metabolism of the tissue investigated and which are seen to be characteristic of all the ionic species that have been considered in this and preceding papers.

Summary

1. The effect of pH and the concentration of a $KHCO_3/H_2CO_3$ buffer on the absorption of bromide and the metabolism of potato discs has been investigated using thin discs in aerated solutions at 23° C.

2. Throughout, the effects of these treatments on the uptake of bromide, on protein synthesis, and on oxidations in the tissue catalyzed by oxidases (phenolase) are closely parallel.

3. At constant pH, increasing the external concentration of $KHCO_3$ and dissolved carbon dioxide depresses (and eventually suppresses completely) both protein synthesis and bromide accumulation. To this effect both combined and free carbonic acid contribute; at pH 7.0, their relative effects are about equal. Of HCO_3^- and undissociated H_2CO_3, the former appears to have the greater effect on the tissue.

4. There is a relatively broad pH range, in the absence of bicarbonate, within which bromide uptake is not much affected by $[H^+]$ and its attendant variables. In the presence of bicarbonate, protein synthesis (though depressed by the salt) is at its maximum at pH 7.0 and is less active in a more

acid or alkaline solution. These effects are specifically due to H^+ and OH^- and find a parallel in bromide uptake which is depressed by bicarbonate solutions and is more favored under these conditions by a pH of 7.0 than by reactions more acid or more alkaline.

5. The effect of HCO_3^- and H_2CO_3 on nitrogen metabolism is also shown by their effect on the utilization of amino acids but it extends to subsequent reactions; *e.g.*, reactions in which unstable amides are involved as intermediaries of protein synthesis.

6. The effects of pH and the concentration of a bicarbonate buffer on the behavior of the tissue (bromide uptake, nitrogen metabolism, etc.) can be represented by 3-dimensional figures. Comparison of these figures shows that bromide uptake and protein synthesis from soluble nitrogen reserves, are similarly affected by these variables.

7. The evidence shows again that in neutral solution (strictly pH 6.9) K^+ and Br^- are equally absorbed by potato discs. At more acid reactions (by CO_2), conditions which are also less favorable for protein synthesis, the tissue loses potassium. The best conditions for protein synthesis are conducive to the maintenance of existing concentrations in the cell sap.

Tissue, however, in which neither bromide uptake nor protein synthesis occurred, absorbed potassium from relatively strong bicarbonate solutions. This special case of potassium absorption (accompanied by HCO_3^- which apparently reacts with the organic acids of the cell sap) is less dependent on the processes of growth than the case in which a cation and a nonreactive anion are absorbed together.

8. Indirect evidence shows that the bicarbonate solutions which retard bromide absorption and protein synthesis also depress respiration and the metabolism of carbohydrate.

The mechanism of the effect of HCO_3^- and H_2CO_3 on metabolism is briefly discussed.

9. The effect of pH and phosphate concentration on respiration and nitrogen metabolism has been investigated using aerated phosphate buffers of constant potassium content.

At pH 7.0, increased concentration of a phosphate buffer increases both respiration and protein synthesis in potato discs.

At constant phosphate concentration *both* respiration and protein synthesis are at a maximum at pH 7.0 and decrease in solutions more acid and more alkaline.

The similarity in the effects of these variables on both respiration and nitrogen metabolism is seen by comparing figures which show simultaneously the variation of the property in question (respiration, protein synthesis, or loss of soluble nitrogen) in relation to two variables (pH and phosphate concentration).

10. Phosphate treatments which stimulate respiration deplete the tissue of the unstable amide, which is normally present in discs of potato in aerated solution and which appears to be a reactive intermediary in protein synthesis.

11. Despite the different specific effects attributable to the phosphate and bicarbonate the parallelism between protein synthesis and respiration is consistent throughout. Although phosphates increase and bicarbonates decrease respiration the effect of pH is similar in both buffer systems. These effects must be ascribed specifically to H^+ or OH^- and, if not due to specific enzymes with definite pH optima, their symmetry about pH 7.0 suggests that they are determined by properties of water and not of the ampholytes (amino acids, proteins) concerned.

Brief reference is made to the general problem of the effect of pH on respiration and to discrepancies in the literature.

12. Phosphates seem to influence metabolism of potato discs through their effect on nitrogen metabolism (protein synthesis and use of amino acids); *i.e.*, processes which appear remote from the accepted rôle of hexose phosphates in carbohydrate breakdown.

13. Respiration, protein synthesis, and bromide absorption are again seen to be closely linked. They are similarly affected by external variables, the effect of which on the tissue is consistently shown by reactions stimulated by the oxidase (phenolase) it contains.

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ACCUMULATION OF ARSENIC IN THE SHOOTS OF SUDAN GRASS AND BUSH BEAN[1]

LEONARD MACHLIS

(WITH SIX FIGURES)

Arsenic, like certain other elements, is toxic to plant and animal life in relatively minute quantities. It has no effect on, or may possibly be beneficial to, plants when present in traces (**3**, **12**, **24**, **25**). Since it is an active poison the extensive use of arsenical herbicides and insecticides has been attended by several hazards.

A small part of the toxicants adheres to the sprayed or dusted fruits and vegetables, thus rendering them inedible unless washed. Legislation now limits the amount of spray residue allowed to remain on these plant products. The greater portion of the applied arsenicals are eventually deposited in the soil where they accumulate in the surface layer (**14**, **17**, **27**) in quantities sufficient to retard plant growth. Unsuccessful crop production because of accumulated arsenical residues has occurred in South Carolina (**1**, **7**), Louisiana (**22**), and Washington (**27**).

In 1937 JONES and HATCH (**17**) suggested that plants growing on arsenated soils might absorb quantities of arsenic which would be deleterious to livestock and humans if such plants were ingested. They found only the normal trace content of arsenic in apples grown on soils high in arsenic as a result of regular spraying practices. The negligible arsenic content of the apples was attributed to the fact that most of the roots were located below the surface layer of the soil in which the accumulated arsenic residues were found.

The investigation reported below was designed primarily to determine the extent of accumulation of arsenic in agricultural plants having access to the element and to assess the data with respect to growers and consumers of plant products.

Data on the arsenic content of plants grown under various conditions are meager. Analyses of plants growing on soils containing naturally-occurring arsenic have shown traces to be present (**16**, **24**, **26**). These are, however, insignificant as well as unavoidable.

Two reports in the literature indicate that high-arsenic soils can produce high-arsenic plants. FELLENBERG (**11**) describes a meadow in the vicinity of Buus, Germany, which he found to contain 700 to 800 times as much arsenic as did cultivated fields in the surrounding country. The plants growing on this soil were also high in arsenic. He writes: "Horses, accord-

[1] Published by permission of the Director of the Hawaii Agricultural Experiment Station as technical paper no. 72.

ing to statements by tenants of Erzmatt, do not eat the hay, and sheep must not be fed with it except mixed with other hay, for otherwise they will become sick. Sheep even have become slightly sick when grazing. Consequently, we are dealing here with amounts of arsenic which already have a toxic effect upon these animals."[2] It should be noted that this soil had a very scanty flora and that attempts to grow different cultivated plants ended in failure. VANDECAVEYE, HORNER, and KEATON (**27**) grew barley plants on soils obtained from the surface six inches of representative areas in each of three fields in the Yakima Valley, Washington. Two attempts to grow crops of barley and alfalfa on two of these soils failed. These had previously been in orchards which had been sprayed with lead arsenate over a period of years. The third had never been in orchard and was productive. The tops from barley plants grown on the unproductive soils yielded 10.01 and 17.50 p.p.m. As_2O_3 respectively, on the dry weight basis; only a trace was found in the plants grown on the productive soil. The plants high in arsenic grew very poorly.

These examples indicate that some plants are capable of absorbing considerable quantities of arsenic from soils which have high arsenic contents. Such soils occur in the cotton belt (**1**, **21**, **22**), orchard growing regions (**14**, **27**), and probably wherever arsenicals have been used over a period of years. They are a potential source of arsenic in plant food products.

From the point of view of this paper, a high arsenic content alone is of no significance. When different plants are grown in increasing concentrations of arsenic of the order of 0 to 25 p.p.m. there is a progressive decrease in growth (measured by weight) up to a lethal concentration which varies with the plant species (**3**). Exceedingly low concentrations of arsenic do not retard growth. For each plant, then, there is a range of concentrations of arsenic in which growth and accumulation of the element may take place. It is clear that good growth and extensive accumulation must take place concurrently if this phase of the general arsenic problem merits serious consideration. Studies were begun in 1937 at this station to obtain information pertinent to the problem. This report presents certain experiments in which water cultures were employed.

Materials and methods

The two plants selected for study were Sudan grass (*Sorghum vulgare* var. *sudanense*) and bush bean (*Phaseolus vulgaris* var. *humilis*). The former is used as hay and ensilage and the latter is raised for its fruit. Each plant was grown in two different but overlapping series of concentrations of sodium arsenite ($NaAsO_2$) which are listed in table I. One series

[2] This quotation and a later one are from a translation for which the writer is indebted to Mr. F. THOMPSON, translator for the Division of Plant Nutrition, University of California, Berkeley.

TABLE I

EFFECT OF THE CONCENTRATION OF ARSENIC IN THE CULTURE MEDIUM ON THE DRY WEIGHT YIELD OF SUDAN GRASS AND BEAN*

Sudan grass series I. Growth period: 63 days					Sudan grass series II. Growth period: 58 days				
Culture concentration of As	Yield of tops	Yield of tops‡	Yield of roots	Yield of roots‡	Culture concentration of As	Yield of tops	Yield of tops‡	Yield of roots	Yield of roots‡
p.p.m.	*gm.*	%	*gm.*	%	*p.p.m.*	*gm.*	%	*gm.*	%
0.0	89.0	100	11.9	100	0.00	50.0	100	9.4	100
1.0	66.1	74	10.6	89	0.25	55.2	110	10.3	110
2.0	49.1	55	10.2	86	0.50	50.1	100	10.1	107
3.0	42.9	48	8.1	68	0.75	47.4	95	9.3	99
4.0	30.9	35	7.1	59	1.00	44.2	88	9.8	105
5.0	35.7	40	8.1	68	1.25	38.5	77	9.5	102
6.0	30.8	35	7.4	62	1.50	43.9	88	9.3	99
7.0	20.3	22	5.4	45	1.75	41.8	83	9.3	99
8.0	25.2	28	6.0	51	2.00	42.3	85	8.8	94
9.0	18.7	21	4.4	37	2.50	35.3	75	8.2	88
10.0	20.2	22	5.0	42	...	...	...	...	...
12.0	11.7	13	3.7	31	...	...	...	...	...
14.0	5.0	5	1.5	12	...	...	...	...	...
16.0	4.9	5	1.3	10	...	...	...	...	...
18.0	0.0	0	0.0	0		...	...	...	...
Bean series I.† Growth period: 4 days					**Bean series II. Growth period: 29 days**				
0.00	11.0	100	...	...	0.0	14.2	100	7.0	100
0.25	6.4	58	...	...	0.1	16.0	112	8.1	116
0.50	4.6	42	...	...	0.2	13.0	92	7.0	99
0.75	2.8	26	...	...	0.3	16.3	114	9.1	129
1.00	2.2	20	...	...	0.4	19.0	131	10.7	153
2.00	0.4	4	...	...	0.5	13.4	95	8.1	116
3.00	−0.7	−6	...	...	0.6	14.2	100	8.2	119
4.00	−0.1	−1	...	...	0.7	9.6	68	6.8	96
5.00	0.0	0	...	...	0.8	7.3	52	5.0	71
6.00	−0.8	−7	...	...	1.0	6.4	45	4.1	55
7.00	−0.3	−3	...	...	1.2	2.2	15	2.7	38
8.00	−0.2	−2	...	...	1.6	0.5	4	1.5	21
9.00	0.7	6	...	...	1.0	0.4	3	1.6	22
10.00	0.7	6	...	...	3.0	0.0	0	1.1	16

* The yields recorded are for a single plant for Sudan grass; 10 plants for bean series I; single plant for the tops of bean series II; and 5 plants for the roots of bean series II.

† The negative values for bean series I are caused by variation in the weights of the seedlings at the beginning of the arsenic treatment.

‡ Percentage of control.

covered the entire range of concentrations in which each plant could live; the second contained a larger number of concentrations in the non-toxic or slightly toxic range. The terms "toxic" and "toxicity" are used in the sense of any repression of growth as measured by the dry weights of the

plants. The arsenic content of the stem, leaf, node, etc., of the Sudan grass plants at several intervals between the seedling and flowering stages and of the bean plants for a single collection was determined.

Each series was grown in duplicate. With the exception of bean series I all data are averages of two independently determined values which did not vary sufficiently to affect the present interpretation. The arsenic content of the root was not systematically determined owing to the difficulty of distinguishing between arsenic held internally and externally.

Culture methods

The plants were grown in 17- and 20-liter crocks on stationary tables in a greenhouse on the University of Hawaii campus. Each crock was fitted with a varnished plywood cover cut to hold six or eight flat corks which, in turn, were bored to hold a maximum of five plants each. Sudan grass seeds were germinated on cheesecloth supported over water in enameled pans and the bean seeds between sheets of moist paper. Selected seedlings, held in the corks with nonabsorbent cotton, were grown in arsenic-free nutrient solution for 7 to 10 days. At the end of this period the nutrients were renewed and the required amounts of arsenic added to each culture from a stock solution of $NaAsO_2$. Both nutrients and arsenic were thereafter renewed weekly for the duration of each experiment. Occasional analyses of the solutions showed that the arsenic concentration decreased slightly during the week from the calculated initial concentrations. Thus, when the latter were 0.25, 1.0, 5.0, 10.0, and 18.0 p.p.m. As, the concentrations at the end of the week were found to be 0.14, 0.72, 4.62, 9.56, and 17.22 p.p.m. As, respectively. When the plants had attained considerable size, suitable support was provided to keep them upright. The environmental conditions were equalized as much as possible by varying the positions of the cultures.

Hoagland's nutrient solution consisting of 0.005 M KNO_3, 0.005 M $Ca(NO_3)_2$, 0.002 M $MgSO_4$, and 0.001 M KH_2PO_4 was used for Sudan grass and a three-salt solution composed of 0.005 M $Ca(NO_3)_2$, 0.002 M $MgSO_4$, and 0.002 M KH_2PO_4 for the bean plants. To both solutions $FeSO_4$, $MnSO_4$, and H_3BO_3 were added in small quantities. The experiments with bean were done following those with Sudan grass. The change to the three-salt solution was made because this nutrient was easier to prepare and produced healthy plants. It is considered unlikely that the changes in the concentrations of the salts were of magnitudes that could affect the interpretation of the data.

These solutions were made up in quantities of 100 liters and transferred to the crocks in a galvanized iron container designed to deliver either 17 or 20 liters of solution. Baker's C.P. grade chemicals, distilled water, and

continuous forced aeration were used in all of the experiments. Normal H_2SO_4 was added as needed to maintain the pH between 4 and 5. The plants were sprayed occasionally with nicotine sulphate (1:800) to control thrips and aphids.

Plan of experiments

Sudan grass series I.—Thirty seedlings, nine days old, were placed in each crock (17-liter capacity) containing arsenic-free nutrient solution on Oct. 12, 1938. One week later the solutions were renewed and the required amounts of arsenic added. On Oct. 26, Nov. 2, 9, and 23, and Dec. 7, and 21, respectively, one cork of five plants was removed from each culture, weighed, and prepared for analysis. The plants were divided into the following parts: Oct. 26 and Nov. 2 collections into root and plant top; subsequent collections into root, tillers, and the primary shoot which was further divided into node, internode, leaf, and inflorescence, if present.

For the purpose of this study the plant top of Sudan grass was divided into the main axis, or primary shoot, and the tillers or secondary shoots that arise from axillary buds at the basal nodes of the main axis. The latter produce their own adventitious root systems. The division was made for two reasons: (1) the tillers are of variable size and consequently difficult to dissect completely into their several morphological parts; and (2) they are produced at all times during the experimental period, whereas the primary shoot is present from the beginning. In the text the tillers plus the primary shoot are referred to as the plant top. The nodes, leaves, internodes, and inflorescences are those of the primary shoot only.

Sudan grass series II.—Forty seedlings, 7 days old, were placed in each crock (20-liter capacity) on Oct. 21. One week later the solutions were renewed and the arsenic added. One cork of five plants was removed from each culture on Nov. 4, 11, 18, and 25, and on Dec. 2, 9, 16, and 24, weighed, and prepared for analysis. The plants were divided as follows: Nov. 4 and 11 collections, into root and plant top; Nov. 18 and 25 into root, tillers, and primary shoot; and the remaining collections into root, tillers, node, internode, leaf, and inflorescence as above. The Dec. 2 and 16 collections were not analyzed for arsenic.

Bean series I.—The plants in this series did not survive in concentrations of arsenic in excess of 2 p.p.m.; consequently, it was discontinued 4 days after the addition of arsenic to the cultures. Five seedlings, 3 days old, were introduced into each crock (17-liter capacity) on Jan. 15, 1939. The solutions were changed and the arsenic added on Jan. 26 and four days later the plants were harvested. The plants from duplicate cultures were composited, and only the shoots prepared for analysis without further division.

Bean series II.—Five seedlings, five days old, were placed in each crock

(17-liter capacity) on Feb. 4. The required amounts of arsenic were added Feb. 16. One month later the plants were harvested, divided into root, stem, petiole, leaf blade, and fruit, and prepared for analysis.

Analytical methods

The fresh plant material was cut into small pieces and dried to constant weight in a blast of hot air at 85° to 95° C. The larger samples were ground in a Wiley mill, the smaller in a drug mill; both were stored in paper envelopes. Prior to analysis, the opened envelopes were placed in an electric oven at approximately 60° C. for six hours or more and then cooled in a desiccator.

The official digestion method used in the Gutzeit procedure (**20**), modified by the substitution of perchloric acid for ammonium oxalate in the driving off of nitrogen oxides (**5**), was adapted to the small quantities of material available. The procedure used was as follows: Two grams or less of the dried ground sample were weighed into 125-ml. Erlenmeyer flasks which had previously been calibrated and marked at the 125-ml. level. To the aliquot, 1 to 2 ml. of H_2O, 20 ml. of HNO_3, and 10 ml. of H_2SO_4 were added in order. These were shaken carefully two or three times over a 15-minute period. When the reaction subsided, the flasks were covered and allowed to stand for several hours to prevent excessive foaming when subsequently heated on an electric hotplate. During the heating 5 to 10 ml. portions of HNO_3 were added to the solutions until, with the evolution of SO_3 fumes, a clear white or amber color obtained. The flasks were then removed from the hotplate, allowed to cool slightly, and 2 ml. of 70 per cent. perchloric acid were added. After this, heating was continued until the evolution of dense SO_3 fumes. They were cooled again and made up to 125 ml. Sixty samples can be digested in this way in about three hours with a sufficiently large hotplate.

In the estimation of arsenic, the titration method of Cassil and Wichmann (**6**) was used with the exception that the maximum quantity of arsenic determined was limited to 50 micrograms As_2O_3. Arsenic is reported as As unless otherwise designated.

The inherent standard deviation of a single analysis was found from quadruplicate analyses of four samples of arsenic-containing plant material. The amounts found (as micrograms As_2O_3 per gram of dry material) and the standard deviations (corrected for small samples) were 17.68 ± 0.26, 26.67 ± 0.70, 32.25 ± 0.58, and 51.68 ± 0.46. The average of the standard deviations over the entire range was ± 1.79 per cent.

Time did not permit a critical determination of the percentage recovery of arsenic over the range used (**6**). Estimates based on limited tests indicate that in aliquots containing from 5 to 50 micrograms As_2O_3 the amount found may be 5 per cent. less than the actual amount and in aliquots con-

taining less than 5 micrograms the amount found may deviate as much as ± 10 per cent. from the actual amount. Affected by the latter error are 15 per cent. of the values presented for the arsenic contents of the plant parts of Sudan grass series I and II and 80 per cent. of the values for the plant parts of bean series II.

Results

Growth of the plants

Yield.—The yield is here considered to be the dry weight of plant material produced in each culture subsequent to the initiation of the arsenic treatment. The data for each series are presented in table I. It should be noted that the yields of Sudan grass, as defined above, represent a summation of the weights of the plants removed for the several collections from any one culture. This method was adopted to equalize variation between the weights of the plants in a culture.

With reference to the plant tops, concentrations of arsenic below 0.5 to 0.6 p.p.m. were non-toxic to both plants and may have been beneficial, while above this the yield was progressively reduced as the arsenic approached the lethal concentrations of 18 and about 2 p.p.m. of As, for Sudan grass and bean, respectively. The roots were similarly affected but in all concentrations they produced relatively more dry material than the tops.

The maximum concentrations of arsenic in which these plants can grow are probably closer to 1.2 and 10.0 p.p.m. for bean and Sudan grass, respectively. The roots of plants growing in concentrations in excess of these were almost immediately killed and were thus non-functional. Under this condition, the water, nutrients, and arsenic would enter directly into the transpiration stream, which probably accounts for the slight growth of the tops observed. Under soil conditions it is unlikely that such plants could have survived. Out of a total of 60 Sudan grass plants in the duplicate cultures containing 12, 14, 16, and 18 p.p.m. As only 53 per cent., 23 per cent., 10 per cent., and 0 per cent., respectively, survived the first week of treatment.

The concentrations, 1.2 and 10 p.p.m. As for bean and Sudan grass, respectively, determine the maximum concentrations of arsenic from which accumulation may take place by each plant. These relative sensitivities are directly applicable to soil conditions and apparently are representative of closely related plant species. Thus Brenchley (3) found that in water cultures 4 p.p.m. arsenious acid prevented the growth of peas and 20 p.p.m. that of barley. Morris and Swingle (18) observed that when several different arsenicals were added to soil plots, beans were killed; barley grew uninjured. Their report also indicated that some differences in sensitivity are to be expected between related plant species. Stewart and Smith (24)

observed that beans seemed decidedly more sensitive than wheat and other plants growing in soil to which disodium arsenate had been added. Peas were more resistant than beans and potatoes, and seemed more sensitive than wheat.

The high yields of some of the plants growing in the very weak concentrations of arsenic, although probably not statistically significant, suggest a beneficial function for arsenic. This may be the indirect effect of the action of the arsenic on the fauna and flora of the cultures or it may indicate the essentiality of the element. Increased growth or healthier appearing plants have been reported where arsenic was added to soil plots (**12, 24, 25**); BRENCHLEY (**3**), however, using water cultures, was unable to find any evidence indicating a beneficial action of arsenic.

If the criteria of ARNON and STOUT (**2**) for essentiality are accepted, none of the experiments, including those reported herein, have demonstrated to date a requirement of plants for arsenic.

OTHER RESPONSES.—The first recognizable response of the plants to the arsenic treatment was a wilting of the leaves of both species. This occurred daily for a period of several days with a recovery of turgescence at night. The extent of wilting of the plants growing in the various concentrations of arsenic was more or less proportional to the toxicity of the arsenic to the plants as indicated in table I and recovery was inversely so. The wilting took place very quickly. For example, arsenic was added to the cultures of bean series II at 1:30 P.M. on a sunny day and by 3:30 P.M. some of the leaflets of the plants growing in the highest concentrations of arsenic were wilting. About 10:00 A.M. the next morning all of the plants in solutions containing 0.5 p.p.m. or more As displayed some degree of wilt. The Sudan grass plants responded in a similar manner.

Two to three days after the introduction of arsenic into the solutions necrotic leaf tissue was observed. The killed portions were the tips of the Sudan grass leaves and the margins of the bean leaves. The extent of killed tissue increased with increasing concentrations of arsenic in the cultures. The daily wilting decreased in severity as the plants became older. After two or three weeks a noticeable wilt was observed only on the day or two immediately following the renewal of the arsenic and nutrients.

The leaf injury took place predominantly during the first week of growth in the arsenic-containing solutions. New leaves produced in all of the Sudan grass cultures and in most of the bean cultures subsequent to the initial injury were for the most part green and healthy. This was noteworthy because, as will be shown later, these apparently healthy leaves contained high concentrations of arsenic.

It was observed that the roots became flaccid—again in proportion to the toxicity of the arsenic to the plant. Those in the very high concentrations

(above 1.2 and 12 p.p.m. As for bean and Sudan grass, respectively) soon became gelatinous and disorganized.

The root systems tended to become red two or three days after the additions of arsenic to the cultures. Those of the bean and Sudan grass plants growing in concentrations of less than 3 p.p.m. of As were tinged a slight pink. The color did not become noticeably more intense with time. The Sudan grass roots in the higher concentrations soon became a dark red, particularly the larger roots, with the exception of the roots tips. The root systems of the bean and Sudan grass plants that were observed to become gelatinous did not become discolored, suggesting that they were killed before the reactions resulting in the reddening could take place. The cortical tissues were most severely affected for they could be removed leaving the white stele with its attached, small, lateral roots.

In brief, it seems that the arsenic effected a sudden decrease in the movement of water into the plants. As a result of this interference with the supply of water to the plant top the leaves wilted and died back. MORRIS and SWINGLE (**18**) working with both water cultures and soils, observed that the addition of arsenicals to the growth media was followed by a marked decrease in transpiration. FELLENBERG's description (**11**) of plants growing on a high-arsenic soil also suggests that arsenic interferes with the water relations of plants.

The reduced yields of bean and Sudan grass may be a response to this physiological drought; however, arsenic was found throughout the plants and could inhibit growth by interference with other processes.

It is significant that no injury or abnormality of the plant tops was observed that was characteristic for arsenic poisoning. The wilting and subsequent killing of part of the leaves, as well as the decreased yield of the plants, apparently differed in no way from similar responses to be expected from a sudden drought.

Analytical data

CONCENTRATION OF ARSENIC IN THE PLANT TOPS

In figure 1 the weighted average concentrations of arsenic found in the plant tops of Sudan grass series I at the time of each collection are presented graphically. It is to be noted that over the range of 0 to 8 p.p.m. As in the culture solutions the concentration of arsenic in the plant top is a straight-line function of that in the nutrient solution and, secondly, that it remains constant with time for each culture. The widely fluctuating values for the plants growing in the high concentrations of arsenic (9 to 18 p.p.m. of which those above 12 are not plotted because the range of values was too extensive to get into the figure) are indicative of the previous observation that the roots of these plants were non-functional.

Similar data for Sudan grass series II are substantially a reproduction of those of series I over the corresponding range of solution concentrations. There was a tendency for the concentrations of arsenic within the plant tops of series II to decrease during the course of the experiment; the magnitudes of these changes were necessarily small, however. Data are presented for the Dec. 9 collection (table V) representing a series of concentrations midway between the highest and lowest observed during the experimental period. These data for series II are to be compared with the Dec. 21 collection of series I (table IV) which is representative of the concentrations found in the plant tops of series I throughout the experimental period.

In a like manner, the concentrations of arsenic in the plant tops of the two bean series are substantially the same for those plants growing in equal concentrations of arsenic even though bean series I was conducted for 4 days and bean series II for 30 days (tables VI, VII).

The values found in each of the four series are compared in table II.

TABLE II

CONCENTRATION OF ARSENIC IN THE PLANT TOPS OF BEAN AND SUDAN GRASS AS PARTS PER MILLION OF ARSENIC, DRY WEIGHT BASIS

Culture concentration	Bean series I	Bean series II	Sudan grass series I	Sudan grass series II	
	4 days	30 days	63 days	42 days	56 days
p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
0.25	3.6			3.9	1.3
0.50	5 2	3.9		4.7	2.9
0 75	3.9			7 4	4 3
1.00	4.3	8.4	9 6	7.8	5.1
2 00	17.9	20.0	14.7	15.6	11.2

The comparison is obviously restricted to those plants growing in equal concentrations of arsenic. Values from two different collections of Sudan grass series II are presented owing to the tendency for the arsenic content of these plants to decrease with time. It is seen that irrespective of the plant species or of the length of the growth period in the arsenic-containing medium the arsenic concentrations in all of the plants are of the same order of magnitude for each concentration of arsenic in the nutrient solutions.

The data indicate that arsenic readily enters the plants. The concentration in the plant top is governed, apparently, by that in the nutrient solution; hence, the sensitivity of the plant to arsenic poisoning determines the maximum concentration of arsenic that it may contain. Of the two plants, Sudan grass is potentially more deleterious to humans or livestock than bean since it can grow in higher concentrations of arsenic and thus contain higher concentrations.

Distribution of arsenic within the plants

The concentrations of arsenic in the parts of the Sudan grass plants at the time of each collection over the range of 1 to 10 p.p.m. are plotted in figures 2 to 5 from data for series I. Similar data for series II repeat the initial portions of the curves of figures 2 to 5. The leaves and nodes contain the highest concentrations, the internodes are intermediate but below the plant top averages; the inflorescences are very low. These differences become more marked with time in series I because of a tendency for the concentration of arsenic in the nodes and leaves to increase, although those of the tillers and internodes show no change. These increases are not reflected in the curves for the plant tops (fig. 1) because they are offset by the appear-

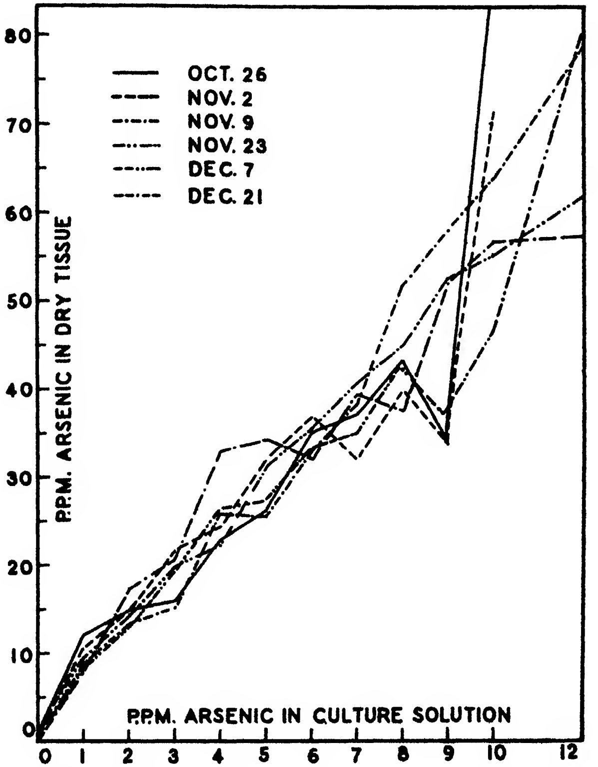
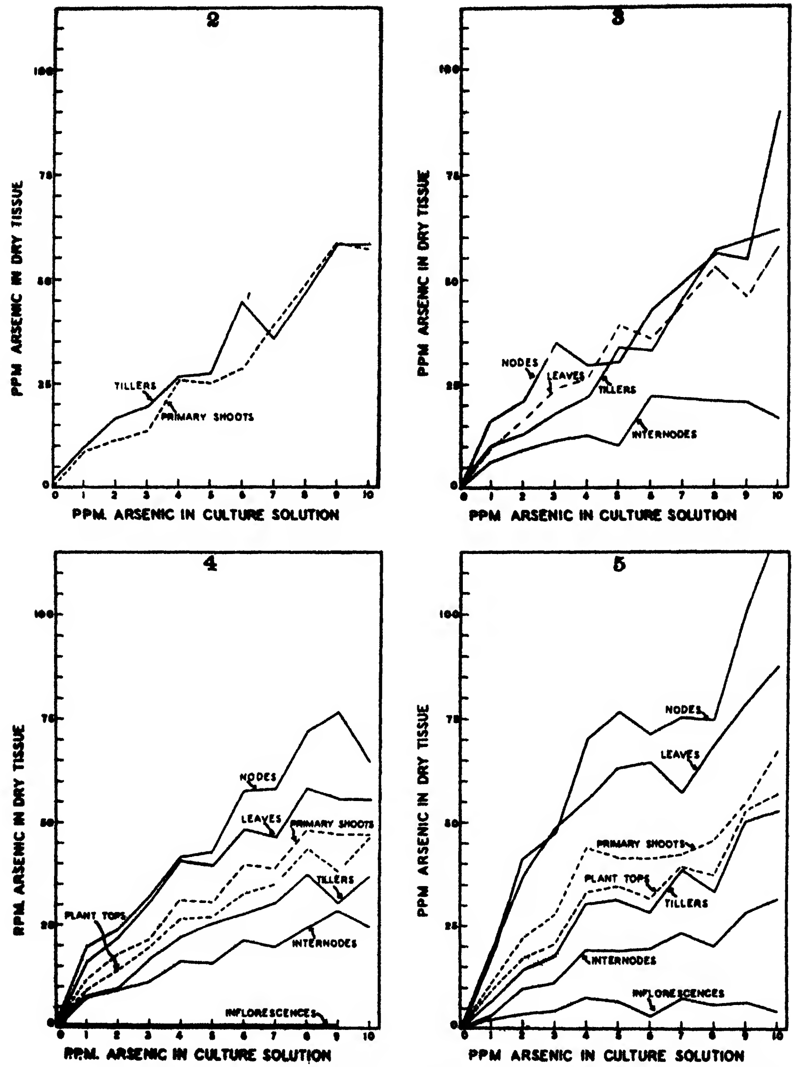


FIG. 1. Concentrations of arsenic in the plant tops of Sudan grass after growing for different lengths of time in culture solutions containing various concentrations of arsenic. The arsenic treatment began on Oct. 19, 1938.

ance of the low-concentration inflorescences and because, as the plants aged, the leaves came to represent a relatively smaller portion of the plant top. The close agreement of the curves for the plant tops, primary shoots, and tillers was to be expected (figs. 5, 6) for each represents an average of the



FIGS. 2–5. Concentrations of arsenic in different parts of Sudan grass after growing for 3 weeks (fig. 2), 5 weeks (fig. 3), 7 weeks (fig. 4), and 9 weeks (fig. 5) in culture solutions containing various concentrations of arsenic.

concentrations of arsenic in the nodes, leaves, internodes, and inflorescences.

The distribution in the bean plant is similar (fig. 6). The leaves are highest, stems and petioles intermediate, and the fruit lowest in concentration of arsenic. Except for the reproductive structures the concentration of arsenic in any specific part of either plant species is a function of the solution concentration.

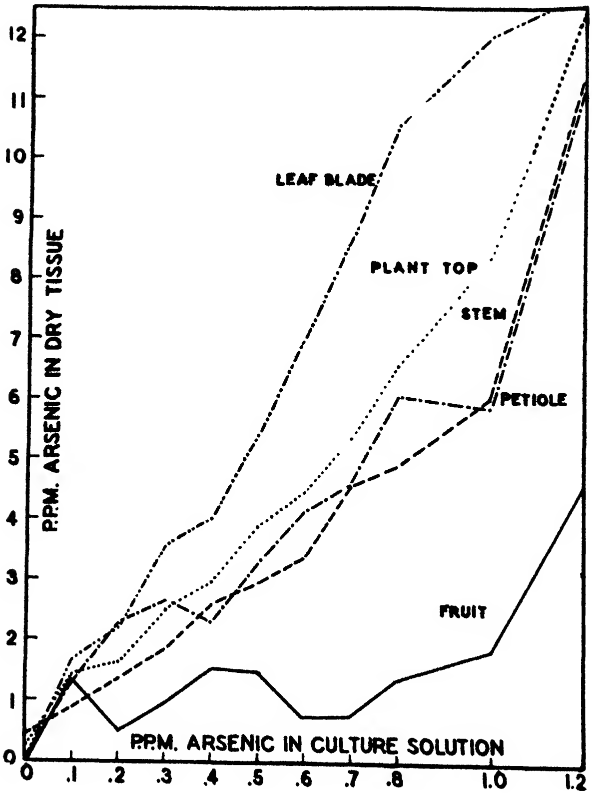


FIG. 6. Concentrations of arsenic in different parts of bean plants grown for 29 days in culture solutions containing various concentrations of arsenic.

The percentage of the total arsenic of the primary shoot of a plant found in any one part of the plant, *i.e.*, the percentage of the total arsenic in the leaves, for example, was the same for all the plants irrespective of the arsenic concentration in the solution. Similarly, the dry weight of each part of the primary shoot relative to the total dry weight of the primary shoot did not vary with the solution concentrations. Averages and standard deviations of these measures are presented in table III. These calculations are based on the Sudan grass and bean plants growing in concentrations of arsenic up to and including 10 and 1.2 p.p.m. As, respectively. Similar calculations made from data from plants growing in concentrations in excess of the foregoing deviated considerably from the averages, a result attributed to the non-functioning root systems of these plants.

The changes with time of the distribution of the weight of Sudan grass are in accordance with expectations based on the nature of the growth of these plants. The decrease in the relative weight of the leaves of the

TABLE III

Distribution of the total arsenic and dry weight of Sudan grass and bean plants among their several parts. Explanation in text

	Dry weight of the plant part as a percentage of that of the primary shoot								Dry weight of primary shoot as a percentage of that of the plant top	
	Leaf		Node		Inter-node		Inflo-rescence			
	%	S.D.*	%	S.D.	%	S.D.	%	S.D.	%	S.D.
Sudan grass series I, Dec. 7	53.8	7.1	8.5	2.7	35.5	4.5	3.2	2.3	45.5	8.1
Sudan grass series I, Dec. 21	41.5	4.8	9.3	0.4	37.1	8.3	11.8	2.3	34.5	5.1
Sudan grass series II, Dec. 9	54.5	3.9	9.2	1.4	35.2	2.8	2.1	1.4	62.8	6.3
Sudan grass series II, Dec. 25	38.4	2.3	8.8	0.8	39.9	2.0	13.1	2.4	37.3	6.2
			(Petiole)		(Stem)		(Fruit)			
Bean series II	49.2	5.7	7.1	0.4	20.3	2.3	23.5	1.9	......	...
	Arsenic content of the plant part as a percentage of that of the primary shoot								Arsenic content of primary shoot as a percentage of that of the plant top	
Sudan grass series I, Dec. 7	68.7	5.3	12.5	9.0	18.8	3.7	......	...	53.4	7.9
Sudan grass series I, Dec. 21	65.8	1.8	16.0	1.4	16.6	1.3	1.7	0.5	42.3	4.9
Sudan grass series II, Dec. 9	73.0	4.4	10.6	2.7	16.6	3.2	... ...	..	73.6	6.4
Sudan grass series II, Dec. 25	66.9	8.1	9.5	1.7	22.6	4.1	3.3	1.7	39.9	8.5
			(Petiole)		(Stem)		(Fruit)			
Bean series II	69.2	8.3	6.5	1.7	16.3	3.7	8.0	6.8	.....	...

* S.D. = standard deviation.

primary shoots is a reflection of the fact that during the last two weeks of growth of both series the primary shoots were entering the reproductive phase of activity and hence were not producing any new leaf or stem tissue although the tillers continued to do so. That the concentrations of arsenic in the solutions or in the plants did not affect these distributions indicates that the arsenic suppressed the growth of the entire plant and not of any paricular morphological part. This suggests an interference with the functioning of some one or more of the fundamental processes of the plants.

The distribution of arsenic is unaffected by either the period of growth in the solutions or the concentrations of arsenic in the solutions. Although both the nodes and leaves were previously observed to contain nearly the same concentrations of arsenic it may be seen in the table that approximately two-thirds of all the arsenic entering the primary shoots of either

Sudan grass or bean is carried to the leaves whereas the nodes contain a relatively small portion of the total arsenic.

The high concentrations of arsenic in the leaves and nodes are of interest because neither of these parts show any macroscopic signs of injury other than the initial desiccation of the leaf tips. When the nodes were split longitudinally for drying, both the nodal plate and nodal meristem were observed to be white and apparently healthy. Apparently the plants can in some manner render the arsenic inactive. The fact that the arsenic exerted a strong toxic action on the roots, which was expressed in one form as a discoloration, is not inconsistent with the above suggestion; after a period as short as one week in the solutions, relatively enormous concentrations of arsenic were found associated with the roots of Sudan grass. Thus, when the solution concentrations were 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16 and 18 p.p.m. of As, the concentrations in or on the roots as p.p.m. As of dry weight were 338, 263, 390, 514, 655, 734, 903, 906, 1026, 1094, 1246, 1386, 1059 and 1461. These concentrations are several times greater than the highest observed values in the leaves or nodes.

Significance of the arsenic contents of the plants

The magnitude of the concentrations of arsenic found in the plants is of importance both in relation to the physiology of the plant and with respect to the health of humans and livestock that could ingest such plants as food. A complete evaluation of their significance with reference to the latter point would require the consideration of at least the following factors: (1), the concentration of arsenic in the plant and in each of its parts; (2), the chemical state of the arsenic; (3), the magnitudes of the quantities of arsenic that may be considered deleterious to mammals; and (4), the potentiality for growth of plants that contain these magnitudes of arsenic.

The Federal Food and Drugs Act limits the quantity of arsenic that may be present in foods as an adulterant to 0.01 grains per pound which is equal to 1.08 p.p.m of As. Foods containing more than this content are subject to seizure. According to law none of the arsenic present in the plants being considered here is present as an adulterant. This limit does not mean that quantities of arsenic less than 1.08 p.p.m. are of no concern. MEYERS *et al.* (**19**) have on several occasions pointed out the injurious effects of small amounts of arsenic in their relation to the public health.

It is probable that all or part of the arsenic in the plants is tied up in organic molecules which may well affect its toxicity to mammals. A statement by WHITE (**28**) of the U. S. Food and Drug Administration, made in relation to the natural arsenic content of marine products, is relevant: "The data are given with the full recognition that the arsenic may (in the marine products) in some cases, be present in organic combinations which render it

TABLE IV

CONCENTRATIONS OF ARSENIC IN DIFFERENT PARTS OF SUDAN GRASS WHEN GROWN IN CULTURE SOLUTIONS CONTAINING VARIOUS CONCENTRATIONS OF ARSENIC. DATA FROM THE DEC. 21 COLLECTION OF SUDAN GRASS SERIES I

CULTURE CONCENTRATION	LEAVES		NODES	INTERNODES	INFLORESCENCES	TILLERS		PRIMARY SHOOTS		PLANT TOPS		YIELD
	DRY WEIGHT	FRESH WEIGHT*	DRY WEIGHT	DRY WEIGHT	DRY WEIGHT	DRY WEIGHT	FRESH WEIGHT	DRY WEIGHT	FRESH WEIGHT	DRY WEIGHT	FRESH WEIGHT	PERCENTAGE
p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	%
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100
1	18.9†	2.7	17.1	3.4	2.1	6.6	1.1	10.5	2.5	8.1	1.5	74
2	36.6	5.2	41.1	9.9	3.6	14.0	2.5	22.0	5.3	17.2	3.4	55
3	48.0	6.9	47.3	11.2	4.6	17.8	3.0	27.9	6.7	20.7	3.9	48
4	55.3	7.9	69.8	19.1	7.8	30.4	5.2	44.0	7.2	33.0	6.6	35
5	63.5	9.1	76.7	19.0	6.8	31.3	4.7	41.8	8.1	34.7	5.6	40
6	64.8	9.3	71.3	19.5	3.3	28.3	4.5	41.6	8.8	32.0	5.4	35
7	56.9	8.1	75.4	23.2	7.5	38.4	5.7	42.3	8.6	39.4	6.4	22
8	68.3	9.7	74.9	20.2	6.0	33.4	5.2	44.9	9.2	37.5	6.4	28
9	78.8	11.3	101.2	28.1	6.4	50.0	8.2	54.9	11.9	52.3	9.7	21
10	87.4	12.5	126.8	31.2	4.5	52.8	8.9	67.2	14.6	56.9	10.3	22

* The fresh weight of the leaves was not determined. These values are estimated from an assumed moisture content of 85.7 per cent.
† Values italicized are in excess of the tolerance limit.

TABLE V

CONCENTRATIONS OF ARSENIC IN DIFFERENT PARTS OF SUDAN GRASS WHEN GROWN IN CULTURE SOLUTIONS CONTAINING VARIOUS CONCENTRATIONS OF ARSENIC. DATA FROM THE DEC. 9 COLLECTION OF SUDAN GRASS SERIES II

CULTURE CONCENTRATION	LEAVES		NODES	INTERNODES	TILLERS		PRIMARY SHOOTS		PLANT TOPS		YIELD
	DRY WEIGHT	FRESH WEIGHT*	DRY WEIGHT	DRY WEIGHT	DRY WEIGHT	FRESH WEIGHT	DRY WEIGHT	FRESH WEIGHT	DRY WEIGHT	FRESH WEIGHT	PERCENTAGE
p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	%
0.00	0.7	0.1	0.0	0.0	0.0	0.0	0.4	0.1	0.3	0.0	100
0.25	*7.2*†	1.0	*3.5*	*3.0*	*2.2*	0.3	*5.2*	0.8	*3.9*	0.6	110
0.50	*7.5*	*1.1*	*5.1*	*2.5*	*3.6*	0.4	*5.6*	0.8	*4.7*	0.6	100
0.75	*10.3*	*1.5*	*12.7*	*4.6*	*5.4*	0.6	*8.3*	*1.2*	*7.4*	1.0	95
1.00	*13.7*	*2.0*	*11.8*	*4.1*	*5.5*	0.6	*9.3*	*1.5*	*7.8*	*1.1*	88
1.25	*19.6*	*2.8*	*14.1*	*4.7*	*8.1*	0.9	*13.8*	*2.0*	*11.2*	*1.5*	77
1.50	*18.4*	*2.6*	*16.5*	*7.5*	*8.7*	1.0	*14.5*	*2.1*	*12.4*	*1.7*	88
1.75	*20.7*	*3.0*	*20.2*	*8.7*	*10.7*	*1.3*	*16.4*	*2.4*	*14.2*	*1.9*	83
2.00	*24.9*	*3.6*	*20.2*	*7.7*	*11.5*	*1.4*	*17.8*	*3.4*	*15.6*	*2.4*	85
2.50	*27.8*	*4.0*	*29.7*	*10.5*	*16.4*	*1.9*	*22.4*	*3.3*	*20.4*	*2.8*	75

* The fresh weight of the leaves was not determined. These values are estimated from an assumed moisture content of 85.7 per cent.
† Values italicized are in excess of tolerance limit.

TABLE VI

Concentrations of arsenic in different parts of bean when grown in culture solutions containing various concentrations of arsenic. Data from bean series II

Culture concentration	Leaves		Petioles		Stems		Fruit		Plant tops		Yield
	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Percentage
_p.p.m._	_p.p.m._	_p.p.m._	_p.p.m._	_p.p.m._	_p.p.m._	_p.p.m._	_p.p.m._	_p.p.m._	_p.p.m._	_p.p.m._	%
0.0	0.37	0.07	0.12	0.02	0.48	0.10	0.07	0.01	0.32	0.05	100
0.1	_1.66_*	0.28	_1.29_	0.15	0.87	0.15	_1.30_	0.12	_1.38_	0.19	112
0.2	_2.17_	0.37	_2.20_	0.25	_1.34_	0.23	0.48	0.04	_1.60_	0.22	92
0.3	_3.56_	0.60	_2.61_	0.26	_1.87_	0.31	0.91	0.08	_2.48_	0.33	114
0.4	_4.00_	0.62	_2.27_	0.26	_3.74_	0.66	0.74	0.08	_3.88_	0.39	131
0.5	_5.39_	0.89	_3.23_	0.33	_2.92_	0.49	_1.47_	0.13	_4.43_	0.43	95
0.6	_7.06_	_1.13_	_4.10_	0.42	_3.33_	0.58	0.73	0.07	_5.36_	0.59	100
0.7	_8.68_	_1.47_	_4.58_	0.46	_4.50_	0.77	0.75	0.07	_6.52_	0.70	68
0.8	_10.56_	_1.91_	_6.01_	0.61	_4.87_	0.84	_1.32_	0.12	_8.35_	0.87	52
1.0	_12.07_	_2.57_	_5.83_	0.60	_6.04_	0.88	_1.80_	0.16	_12.33_	_1.20_	45
1.2	_13.66_	_3.98_	_11.04_	_1.24_	_11.06_	_1.37_	_4.45_	0.70	_12.06_	_2.39_	15

* Values italicized are in excess of the tolerance limit.

somewhat less toxic than when in the form of lead, calcium, or other arsenates. It is doubtful, however, whether any toxicologist would assert that there is any published work to prove that the natural arsenic in marine and other foods is without any harmful effect.'' This statement indicates that even the smallest quantities of arsenic in the plants are significant. The same reasoning applies to the effects of arsenic on livestock and to the rôle of arsenic in meat food products.

For the purpose of this report the tolerance limit of 1.08 p.p.m. of As will be used as a test of significance. The arsenic concentrations in bean and Sudan grass on the fresh and dry weight bases for representative collections of plants are tabulated in tables IV, V, VI, and VII. Values italicized are in excess of the tolerance limit. These data show that:

TABLE VII

CONCENTRATIONS OF ARSENIC IN THE PLANT TOPS OF BEAN WHEN GROWN IN CULTURE SOLUTIONS CONTAINING VARIOUS CONCENTRATIONS OF ARSENIC. DATA FROM BEAN SERIES I

CULTURE CONCENTRATION	DRY WEIGHT	FRESH WEIGHT	YIELD
p.p.m.	*p.p.m.*	*p.p.m.*	%
0.00	*2.5*†	0.3	100
0.25	*3.6*	0.5	58
0.50	5.2	0.7	42
0.75	*3.9*	0.5	26
1.00	*4.3*	0.6	20
2.00*	*17.9**	*2.1**	4*
3.00*	*27.3**	*3.7**	–6*
4.00*	*32.2**	*4.4**	–1*
5.00*	*35.7**	*4.8**	0*
6.00*	*72.9**	*9.8**	–7*
7.00*	*99.5**	*13.4**	–3*
8.00*	*91.5**	*12.4**	–2*
9.00*	*100.6**	*13.6**	6*
10.00*	*110.9**	*15.0**	6*

* These plants were immediately killed upon the addition of arsenic to the solutions as evidenced by the fact that they made no growth whatsoever. The negative values are caused by variation in the weights of the seedlings at the beginning of the treatment.

† Values italicized are in excess of the tolerance limit.

1. On the dry weight basis, all of the plants and each of their parts exceeded the limit with the exception of the fruit and stem of bean. Particularly significant are the concentrations in those plants whose *yield was not lowered* by the arsenic.

2. On the fresh weight basis, only the leaves of plants whose *yield was unaffected* by the arsenic exceeded the tolerance limit. Sudan grass plants whose yields were slightly decreased exceeded the tolerance limit in all categories whereas a similar result obtains with bean only with an almost complete suppression of growth.

These results follow logically from the interaction of previously observed factors: (1), arsenic is accumulated in the plant tops in concentrations determined primarily by the concentrations in the nutrient solutions, irrespective of the plant species; and (2), the toxicity of the arsenic to the plants is a function of the particular plant species, Sudan grass being some 8 to 10 times more resistant than bean. Thus the more resistant species (Sudan grass) is able to grow under conditions conducive to higher concentrations of arsenic within itself than is the more sensitive species (bean). For equal relative reductions in yield the more resistant species contains several times the concentration of arsenic in the sensitive species. Furthermore, the leaves contain markedly higher concentrations of arsenic than the plant top as a whole, a factor of significance if they are to be used alone as food.

Discussion

In the introduction it was suggested that the gradual accumulation of arsenic in soils as a result of the extensive use of arsenicals for the control of insects and weeds was a possible source of arsenic in foods through its absorption by the plant. The data presented have demonstrated that exceedingly minute quantities of arsenic in the nutrient medium give rise to concentrations of arsenic in the plant tissues that cannot be ignored. Although the toxicity of arsenic and its accumulation from a soil medium may differ substantially in both magnitude and nature from that observed here, arsenic in the soil must be considered as a source from which it can be absorbed in significant quantities by plants, until otherwise demonstrated. Certain combinations of plant, soil, and arsenical would seem to be particularly conducive to the development of high arsenic content plants.

The two classes of arsenicals in general use today are the insoluble arsenates as insecticides and the soluble arsenites as herbicides. They differ in their effects on plants and in their histories in the soil.

Pentavalent arsenic is decidedly less toxic than trivalent arsenic (**3, 4, 18**). Whereas 10 p.p.m. of As in the form of sodium arsenite are lethal to tomato, the same plant can withstand over three times this concentration of arsenic in the form of sodium arsenate (**15**). Absorption may thus take place over a corespondingly wider range of concentrations of pentavalent arsenic than trivalent.

In the soil, absorption of either form is restricted to the available arsenic which is presumably that in solution but may include part of the insoluble or fixed arsenic. ROSENFELS and CRAFTS (**23**) have obtained data suggesting that part of the insoluble or fixed arsenic may be available to the plant. The available arsenic bears no direct relationship to the total arsenic of a soil because soils vary widely in the rate and degree of fixation of arsenic. The effect of the composition of the soil on the concentration of available arsenic

as measured by its toxicity to biological indicators has been studied for arsenites by CRAFTS and his co-workers (**8, 9, 10, 23**), and for arsenates by GILE (**13**), STEWART and SMITH (**24**), PADEN and his associates (**7, 21**), and others. These studies indicate that, in general, the heavier the soil the greater is its ability to fix the arsenic. Conversely, leaching of arsenic tends to be more rapid from lighter soils. An exception is the ferruginous soil type which has a very high fixation capacity for arsenic.

When insoluble arsenates are added to the soil they are, of course, at first unavailable to the plant. Under the action of the soil solution, however, they are partially dissolved (**24**). As the total arsenic of the soil is increased by annual additions of the arsenical, the available fraction should increase. Because of their low fixation capacity, light soils would tend to become toxic with fewer applications of the arsenical than would heavy soils. It is the lighter soils that have become unproductive in South Carolina (**1**) as a result of annual applications of calcium arsenate in the control of the cotton boll weevil. Leaching must obviously have taken place at a slower rate than had accumulation in this soil. Numerous soil analyses indicate that most of the added arsenicals are retained in the upper few inches (**14, 17, 27**). These insoluble arsenicals thus constitute a long-time reserve which, under the influence of the soil solution, is slowly made available to the plant.

The soluble arsenicals, upon addition to the soil, are immediately available in high concentrations. More or less of the arsenical is then fixed to an extent and at a rate determined by the soil type. Two possible undesirable consequences may result from the use of herbicides: (1) an unusually heavy application may so increase the available arsenic content of the soil for a short time as to allow appreciable absorption and perhaps cause injury to the plants; (2) annual applications can build up a reserve of fixed arsenic which will function similarly to the insoluble arsenicals. If the fixation capacity of the soil is exhausted, then further application would be very potent until leached away.

What appears to be an example of injury from the unrestricted use of a soluble arsenical was observed during the course of this investigation. An herbicide was applied to the underbrush of an orchard of macadamia nut trees at Honokaa, Hawaii. Sometime later some of the trees became sickly. It was noted that many of the roots of the trees were either exposed or very near to the surface of the soil. Samples of the roots of diseased and adjacent healthy trees were obtained by first cutting away the surface layers. These and samples of the leaves were analyzed for arsenic with results as shown in table VIII. The roots of the diseased trees contained much higher concentrations of arsenic than did those of adjacent healthy ones.

Because arsenicals tend to accumulate in the upper layer of the soil.

TABLE VIII

CONCENTRATIONS OF ARSENIC (AS As_2O_3) IN THE ROOTS AND LEAVES OF DISEASED AND HEALTHY MACADAMIA NUT TREES*

Diseased trees			Adjacent healthy trees		
Tree no.	Roots	Leaves	Tree no.	Roots	Leaves
	p.p.m.	*p.p.m.*		*p.p.m.*	*p.p.m.*
A-41-13	18.8	8.2	A-41-14	9.3	7.3
A-16-31	29.2	24.1	A-16-32	1.3	5.7
A-10-13	11.5	6.4	A-40-12	6.4	6.3

* The samples were collected and the observations made by the Department of Soil Chemistry, University of Hawaii Experiment Station.

shallow-rooted plants are most likely to accumulate arsenic, as well as to be injured by it.

Finally, plants that are relatively resistant to arsenic poisoning are those capable of growing successfully and, at the same time, of accumulating excessive quantities of arsenic. Such plants would give no indication of their arsenic contents if they responded in a manner similar to bean and Sudan grass in nutrient cultures. It also follows from this investigation that plants which are predominantly leafy, such as spinach and lettuce, may attain very high concentrations of arsenic.

Summary

When Sudan grass (*Sorghum vulgare* var. *sudanense*) and bush bean (*Phaseolus vulgaris* var. *humilis*) were grown for varying lengths of time in nutrient solutions containing various concentrations of $NaAsO_2$, it was found that concentrations of arsenic up to 0.5 to 0.6 p.p.m. had no effect on the growth of either plant as measured by increases in dry weight. Growth was effectively suppressed, however, by concentrations of 1.2 and 12.0 p.p.m. of As for bean and Sudan grass, respectively. Addition of arsenic to the solutions caused an immediate plasmolysis of the roots and wilting of the leaves followed by discoloration of roots and necrosis of leaf tips and margins, indicating that the arsenic effected a sudden decrease in the movement of water into the plants.

The concentrations of arsenic in the plant tops and in each of their parts except the reproductive structures were found to be proportional to the concentrations in the nutrient solutions. The magnitudes of these concentrations were more or less constant during the experimental periods, the longest of which was nine weeks. The leaves were highest, the stem tissues intermediate, and the reproductive structures lowest in concentration of arsenic.

Within a narrow range of concentrations of arsenic in the nutrient medium both plants grew successfully; but they contained concentrations of arsenic in their plant tops in excess of the tolerance limit (1.08 p.p.m. of As)

established by law for the arsenic content of foods. For equal relative reductions in yield the more resistant species (Sudan grass) contained several times the concentration of arsenic in the sensitive species (bean).

Accumulated arsenical residues in soils as a result of insect and weed control practices are discussed as a source of arsenic contamination in foods through absorption by plants.

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WATER UPTAKE AND ROOT GROWTH AS INFLUENCED BY INEQUALITIES IN THE CONCENTRATION OF THE SUBSTRATE

FRANK M. EATON

(WITH FIVE FIGURES)

Introduction

When a number of solutions of unlike concentration are presented at one and the same time either to an established or to a developing root system, questions follow as to whether water is withdrawn as rapidly from the concentrated as from the dilute regions and as to whether new roots extend themselves as readily in the regions of high salinity as in those of low salinity. The subject is of agronomic, as well as physiological, significance since the productivity of soils must be regarded as being contingent not only on their nutrient and water storage resources but also upon the readiness with which these resources can be utilized by plants.

A substantial diversity is customarily found in the salt concentrations of the soil solution within the root zone of single plants growing on irrigated lands. The data of table I, which are presented as an illustration, represent

TABLE I

VARIABILITY OF CHLORIDE, SULPHATE, AND NITRATE CONCENTRATIONS (MILLEQUIVALENTS PER LITER) IN DISPLACED SOIL SOLUTIONS OF A WELL DRAINED BUT SPARINGLY IRRIGATED ORANGE ORCHARD IN THE SIERRA MADRE FOOTHILL AREA OF SOUTHERN CALIFORNIA. SAMPLES WERE COLLECTED ON THE NORTHWEST AND NORTHEAST SIDE OF EACH OF TWO TREES

DEPTH	TREE 30, ROW 22						TREE 36, ROW 22					
	SO_4		Cl		NO_3		SO_4		Cl		NO_3	
	NW	NE	NW	NE	NW	NE	NW	NE	NW	NE	NW	NE
feet	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
0– 3	2.3	3.3	1.2	2.4	4.5	5.0	9.1	2.1	1.6	1.1	2.3	2.0
3– 6	11.8	36.6	1.8	25.2	0.9	4.8	53.3	40.3	13.1	12.1	9.6	8.1
6–10	17.2	40.4	4.2	81.6	4.1	10.5	43.5	42.6	47.9	16.5	25.7	10.2

analyses of displaced soil solutions from samples collected in a sparingly irrigated but relatively permeable and well drained orchard soil. Conductivity measurements made on the solutions displaced from the separate one-foot horizons before combining them for analyses showed a greater variability than is here indicated; the variability in more heavily irrigated soils, however, is usually lower than that found in this instance. All of the

samples were wetted to approximately the moisture equivalent before displacement.

The experimental work here reported upon includes a series of tests conducted in water cultures with tomato and corn plants. The root systems of these plants were divided between solutions that differed in concentration and in the character of the added salts. The effects of the treatments were measured in terms of root growth and water uptake.

Review of literature

The review of literature that follows is confined to papers concerned primarily with the cross transfer of water and solutes in plants. The problem under consideration, however, bears also upon broader fields of inquiry that concern some of the forces involved with water uptake and with some of those governing the growth of roots.

GILE and CARRERO (8) found the total amount of nitrogen or phosphorus absorbed by rice plants having half of their roots in complete nutrient solution and half in solutions deficient in these elements was 76 per cent. of that of plants having all roots supplied with complete nutrient solution. In a subsequent paper (9) they reported a lesser growth of the portion of the root system of corn plants which extended into solutions deficient in nitrogen, phosphorus, or potassium. Their solutions were all dilute (about 5 m.e. of total bases per liter), so there could have been but little difference in osmotic concentrations. MCMURTREY (14) using a similar technique, reported an unilateral development of the leaves of tobacco plants with roots divided between complete nutrient and element-deficient nutrient solutions. He mentioned, and one of his photographs shows, increased root growth when calcium (previously omitted) was added to one of the two solutions supplied to a single plant.

AUCHTER (1) applied nitrate of soda on one side of field trees and concluded that under normal conditions mineral nutrients absorbed by roots on one side are translocated to and used by the trunk, limbs, and leaves above them and that foods manufactured by one side are used or stored on that side or translocated to the roots of the same side. The behavior of trees with half of their roots severed prompted the conclusion that water can move laterally in trees without much difficulty. FURR (7), finding the volume of lemon fruits to be independent of position over cut and uncut roots, also concluded that there was a ready cross-transfer of water throughout old lemon trees. AUCHTER and SCHRADER (2), in experiments continued for two years, thought there was little if any cross transfer of carbohydrates from sun-exposed to cloth-enclosed sides of apple trees. MILLER (15), has reviewed a series of papers showing a tendency toward increased root development in fertilized portions of soils. CALDWELL (4) reports an asymmetric develop-

ment of the fleshy root of the Swede turnip when leaves were removed from one side. The roots below the defoliated side of Coleus plants (5) grew less than the roots under the foliated side. Experiments (1, 5) on plants with roots divided between rich soil and sand have added support to the foregoing conclusions. NIXON[1] has found that date fruits on strands subtended below, and in line with, a notch cut in the fruit stalk that severed about 50 per cent. of the vascular system increased in dry matter during the summer; this indicates some cross-transfer of manufactured foods from the unsevered to the severed side of the stalk.

Methods

Both corn and tomato plants were used for the experiments. The former was regarded a nearly ideal test plant since its vascularity is such as to largely preclude a unilateral development of the top or a differential accumulation of salt in the leaves of one side. It will be recalled that each leaf sheath of the corn plant encircles the stalk at the node from which it arises and that, owing to anastomoses, the nodal structures favor lateral distribution of constituents rising or descending through the vascular system. Anatomical studies of the vascular system of the potato would imply that some lateral distribution of solutes rising or descending in tomato shoots is likewise to be expected.

Lacquered rectangular metal cans with openings cut in one corner, or in the middle of one side of the top, large enough to admit a group of roots were used as culture vessels. These containers (figure 5) used in groups of two or four, were bound together in such a manner that portions of the root system of a single plant could be extended into each. Water was added to the cans through openings having screw caps. Gallon containers were used in experiments 1 and 2, and quart containers in the other experiments.

The plants were grown to a height of 15 to 25 cm. in nutrient solutions in preparation for experimental use and the separation of roots into groups. After dividing the roots into 2 or 4 equal groups, trimming when necessary, they were dusted heavily with carbon black to facilitate a differentiation at the end of the experimental period between the original roots and those subsequently developed.

The plants were fastened in place with cork and cotton mountings, and the roots at the point of division were separated by cork wedges 1 cm. tall that rested on the edges of the cans. Further, to make unlikely the movement of solution from one container to another by wick action, the levels of the solutions were not brought nearer than 1.5 cm. to the tops of the containers. A can with a similar opening was set up in conjunction with each experiment to measure direct evaporation. The loss from this container has been deducted from all results to give net water-uptake values.

[1] R. W. NIXON, unpublished data.

The base nutrient solution contained calcium nitrate, potassium nitrate, magnesium sulphate and potassium dihydrogen phosphate in concentrations of 5, 5, 2 and 1 millimoles per liter respectively (HOAGLAND's solution). This solution was used in the foregoing concentration and in fractional and multiple concentration both alone and in conjunction with added chloride and sulphate salts. These latter salts were added 50 per cent. as m.e. of sodium, 25 per cent. as magnesium, and 25 per cent. as calcium. All solutions contained, in addition to the foregoing, 1 p.p.m. of boron, 0.2 p.p.m. of zinc, and 0.5 p.p.m. of manganese. Iron was added in the form of tartrate or citrate as needed.

The freezing point depressions, $\Delta°$ C, and electrical conductance, $K \times 10^{-5}$, and other determinations of concentration on used solutions were always made after the containers had been restored to their initial weights by adding distilled water. Distilled water was added frequently throughout the experiments to replace transpiration losses. All of the experiments were conducted in a greenhouse. Only the fresh weights of roots are reported since the concentration of the solutions did not affect significantly the percentage of moisture in the roots in those instances (experiment 3) when both fresh and dry weights were measured.

Results

EFFECT OF UNILATERALLY SUPPLIED SALTS ON ROOT GROWTH AND WATER UPTAKE. EXPERIMENTS 1 TO 7

In experiments 1 to 7, inclusive, the root systems of corn and tomato plants were divided between two or more solutions that differed in composition and concentration. The results of these experiments (tables II to V) are alike in showing a greater water-uptake from, and a greater root growth in, the solutions of low concentration than in those of high concentration.

In experiment 1 with corn (table II) nearly twice as great a weight of roots developed in a solution with a trace of chloride as in a solution with 10 m.e.; little or no root growth resulted in solutions with 50 and 250 m.e. of chloride (fig. 1). The effect on water-uptake was also marked. The solution with 250 m.e. of chloride, if it were the only one supplied, would probably be too saline for the growth of corn plants. This relation would not hold for 50 m.e. of chloride since corn plants on such solutions under similar conditions (6) made 62 per cent. as much total growth as in control solutions without chloride. The roots in the chloride solutions of those experiments were heavier relative to the weight of the shoots than in the control solutions.

Chloride determinations (table II) on the solutions at the beginning and end of experiment 1 indicate some movement of chloride through the roots into the solution that started with a trace of chloride.

In experiment 2, with a tomato plant, a significant reduction in water

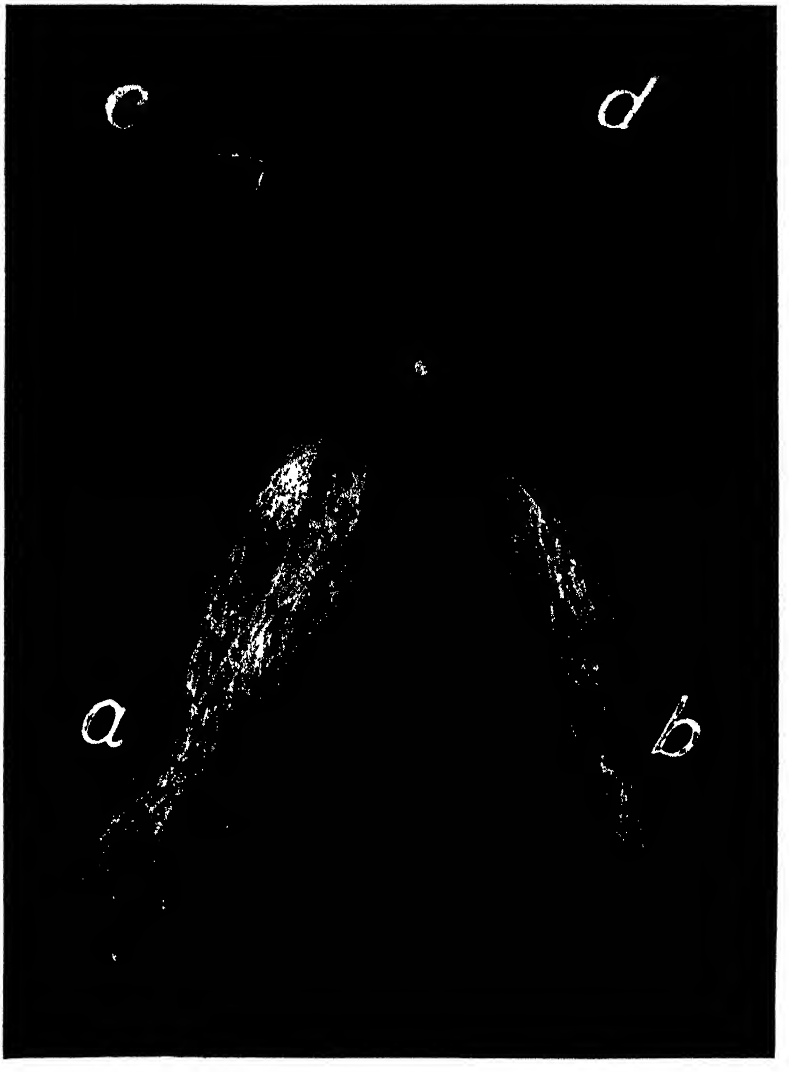

FIG. 1. The differential development of new roots of a corn plant 22 days after the initial roots had been divided between vessels containing in addition to the base nutrient solution: a, trace; b, 10 m.e.; c, 50 m.e.; and d, 250 m.e. of chloride. The roots on the plants at the start of the experiment were dusted with carbon black. Experiment 1.

uptake and a lesser development of roots resulted when the roots were supplied with 22.5 m.e. of chloride; the effects of 7.5 m.e. of chloride, however, were negligible (table I). In this as well as in subsequent experiments with tomatoes, the compact matting of the roots made it difficult to differentiate very accurately between the old and new ones.

TABLE II

ROOTS OF CORN AND TOMATO PLANTS DIVIDED BETWEEN FOUR SOLUTIONS WITH DIFFERENT CONCENTRATIONS OF CHLORIDE. EXPERIMENTS 1 AND 2

CONTAINER	SOLUTION DESIGNATION	K × 10^{-5} AT 25° C.		CHLORIDE PER LITER		NEW ROOTS		WATER UPTAKE	
		INITIAL	END	INITIAL	END				
				m.e.	*m.e.*	*gm.*	*%*	*ml.*	*%*
Experiment 1. Corn plant in base solution with added chloride. Duration 22 days									
A	Trace Cl	220	210	0.10	2.14	8.8	63	908	66
B	10 m.e. Cl	320	298	10.17	9.69	5.1	36	348	26
C	50 m.e. Cl	705	691	48.21	47.50	0.1	1	53	4
D	250 m.e. Cl	2542	2486	254.0	244.0	0.0	0	55	4
Experiment 2. Tomato plant in base solution with added chloride. Duration 22 days									
A	Trace Cl	214	166	0.05	0.11	8.1*	31	820	35
B	7.5 m.e. Cl	291	241	7.46	7.07	8.2*	31	777	33
C	22.5 m.e. Cl	443	392	22.27	21.22	5.4	21	540	23
D	75 m.e. Cl	937	891	73.56	71.64	4.4	17	215	9

* The new and old roots in these containers were so badly matted together that it was impossible to differentiate very closely between them.

All of the original solutions of experiment 2 contained 15 m.e. of nitrate and 6 m.e. of potassium per liter. Measurements made at the end of the test showed that the concentrations in containers A, B, C and D had been reduced to 10.7, 10.9, 11.6, and 13.8 m.e. of nitrate, and to 4.2, 4.7, 4.9, and 6.2 m.e. of potassium per liter, respectively. It is probable that the greater number of roots in the solutions of lower concentration had as much to do with the

TABLE III

CORN ROOTS DIVIDED BETWEEN 4 SOLUTIONS WITH THE BASE NUTRIENT AND DIFFERENT CONCENTRATIONS OF CHLORIDE. DURATION 28 DAYS. EXPERIMENT 3, DUPLICATE TESTS

CONTAINER	SOLUTION DESIGNATION	NEW SOLUTIONS		END SOLUTIONS K × 10^{-5} AT 25° C.	NEW ROOTS		WATER UPTAKE	
		Δ	K × 10^{-5} AT 25° C.					
		°C.			*gm.*	*%*	*ml.*	*%*
Plant no. 1								
A	Trace Cl	0.016	215	55.9	16.5	33	2291	39
B	10 m.e. Cl	0.091	317	158.0	14.7	29	1941	33
C	20 m.e. Cl	0.123	420	280.0	11.6	23	1173	18
D	40 m.e. Cl	0.180	619	504.0	7.3	15	706	10
Plant no. 2								
A	Trace Cl	0.016	215	67.3	9.3	29	2062	37
B	10 m.e. Cl	0.091	317	222.0	8.7	27	1790	32
C	20 m.e. Cl	0.123	420	306.0	7.2	22	1090	18
D	40 m.e. Cl	0.180	619	502.0	7.2	22	827	13

greater uptake of these nutrient ions as did the lower concentrations of chloride, calcium, magnesium, and sodium.

Experiment 3 (table III) included duplicate tests with corn grown in solutions containing, respectively, a trace, 10, 20, and 40 m.e. of chloride. The plants showed depressed root growth and lower water-uptake from the successively stronger solutions. A relatively greater effect on water uptake than on root growth resulted (fig. 2).

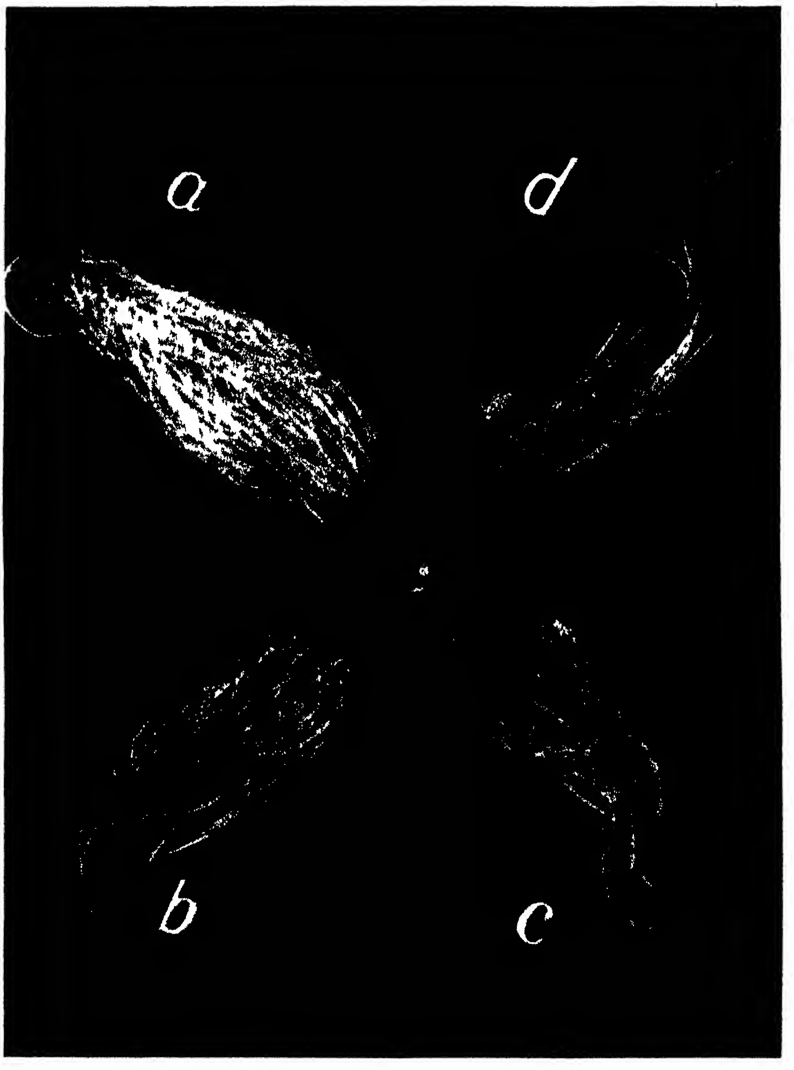

FIG. 2. Roots of a corn plant divided between solutions with: a, trace; b, 10 m.e.; c, 20 m.e.; and d, 40 m.e. chloride. Plant 1, Experiment 3.

Experiments 4 and 5 were conducted for the purpose of determining if sulphate would have an effect similar to chloride on root growth and water uptake. The chloride and sulphate concentrations were selected to give roughly similar differences in the osmotic pressures of the solutions compared. The resulting data (table IV) indicate no pronounced difference between the effects of chloride and sulphate salts at similar osmotic pressures.

TABLE IV

CORN PLANTS WITH ROOTS DIVIDED BETWEEN SOLUTIONS WITH ADDED CHLORIDE (EXPERIMENT 4), AND ADDED SULPHATE (EXPERIMENT 5)

CONTAINER	SOLUTION DESIGNATION	NEW SOLUTION		NEW ROOTS		WATER UPTAKE	
		Δ	$K \times 10^{-5}$ AT 25° C.				
		°C.		gm.	%	ml.	%
Experiment 4. Corn plant with roots in base nutrient with 2 chloride concentrations. Duration 13 days							
A	10 m.e. Cl	0.090	317	2.7	69	306	72
B	25 m.e. Cl	0.138	472	1.2	31	119	28
Experiment 5. Corn plant with roots in base nutrient with 2 sulphate concentrations. Duration 13 days							
A	20 m.e. SO_4	0.097	359	3.6	78	294	68
B	40 m.e. SO_4	0.150	561	1.0	22	139	32

TABLE V

CORN AND TOMATOES WITH ROOTS DIVIDED BETWEEN 3 × BASE NUTRIENT AND 0.5 × BASE NUTRIENT WITH ADDED CHLORIDE. EXPERIMENTS 6 AND 7

CONTAINER	SOLUTION DESIGNATION	NEW SOLUTIONS		NEW ROOTS		WATER UPTAKE	
		Δ	$K \times 10^{-5}$ AT 25° C.				
		°C.		gm.	%	ml.	%
Experiment 6. Corn plant with roots in concentrated base nutrient and dilute base nutrient plus chloride. Duration 13 days							
A	3 × base nutrient	0.157	583	1.8	51	146	54
B	0.5 × base nutrient plus 50 m.e. Cl	0.183	626	1.7	49	125	46
Experiment 7. Tomato plant with roots in concentrated base nutrient solution and dilute base nutrient solution plus chloride. Duration 17 days							
A	3 × base nutrient	0.167	599	4.1	45	435	53
B	0.5 × base nutrient plus 50 m.e. Cl	0.163	534	5.1	55	425	47

Experiments 6 and 7 (table V) were conducted to give further information on the question of whether the previously observed effects were the result of the action of specific ions or primarily reactions related to the total osmotic pressures of the solutions. In these experiments the behavior of corn and tomato plants was observed when their roots were divided between two solutions similar with respect to osmotic pressure but unlike in that one was concentrated base nutrient and the other dilute nutrient plus enough chloride to produce a similar total concentration. The effects of the two solutions were much alike with both corn and tomato. This finding may warrant the conclusion that osmotic relations are primarily involved but, if so, the fact should not be overlooked that nitrate was the principal anion (45 m.e.) in the 3 × base nutrient.

SEPARATION OF EFFECTS OF ROOT AREA AND OSMOTIC PRESSURE ON WATER UPTAKE

At the beginning of each of the foregoing tests the number of roots in the several solutions of an experiment were nearly the same whereas by the end of the experiments there were more roots in the dilute than in the concentrated solutions. Experiments 8 and 9 (table VI) were undertaken to

TABLE VI

DIFFERENTIATION OF EFFECTS OF NEW ROOT DEVELOPMENT AND OSMOTIC PRESSURES ON WATER UPTAKE. EXPERIMENTS 8 AND 9

CONTAINER	SOLUTION DESIGNATION	NEW SOLUTION Δ	NEW SOLUTION $K \times 10^{-5}$ AT 25° C.	NEW ROOTS		WATER UPTAKE	
		°C.		gm.	%	ml.	%
Experiment 8. Corn plant with roots in different concentrations of base nutrient solutions. Duration 13 days							
A	0.5 × base nutrient	0.025	113	3.6	62	451	76
B	3.0 × base nutrient	0.153	581	2.2	38	146	24
Experiment 9. Same as experiment 8 but positions of roots alternated between the two solutions every second day							
A	0.5 × base nutrient	0.025	113			319	65
B	3.0 × base nutrient	0.153	580			171	35

obtain an appraisal of the effects of solution concentration on water uptake that would be independent of number of roots. The first of these experiments (number 8) was conducted in the same manner as those that had pre-

ceded it; in experiment 9, however, the positions of the roots in the two solutions were reversed each second day; *i.e.*, the roots in container A were placed in container B and vice versa. By thus alternating the two halves of the root system between the concentrated and dilute solutions it was possible to maintain essentially equal root areas in the two solutions throughout the experimental period. At the end of experiment 9 one group of roots weighed 32 and the other 36 grams; this is probably as close an agreement as could be expected when account is taken of the difficulties attached to dividing the roots of a plant at the beginning of a test period into two equal groups.

A three-fold difference in water-uptake resulted in experiment 8 where the combined effects of differences in root area and in solution concentration were represented. In experiment 9, however, where essentially equal root areas were presented in each of the solutions, there was approximately a two-fold difference in water uptake. This latter result, which can be regarded as representing the effect of solution concentration alone, will be considered further in the discussion and in figure 5.

Roots divided between nutrient solutions and distilled water

In the three succeeding experiments, tables VII and VIII, the roots of corn and tomato plants were divided between distilled water and 2 × base nutrient. The situation thus created is obviously complex since it is well known that distilled water is injurious to plant roots. The question remained, nevertheless, as to whether the injury to roots would be of such a character as to lessen the rate of water uptake and also whether there would be enough movement of salts from the roots to the distilled water to permit some growth after several days had elapsed.

True (**18**) has traced the development of views on the causes of injurious effects of distilled water on root growth from the observations of early physiologists, who thought the absence of nutrients was responsible, through the period when the ill effects were attributed to metals derived from stills. From his own work with lupine seedlings (**18**), he concluded that a causal connection between loss of electrolytes by roots and their falling growth rate was almost certain. Injury was not prevented in his experiments when sugar or sodium chloride was added to the solutions to give osmotic pressures equal to that of Potomac River water used as a control. Calcium chloride prevented injury. True, referring to work of True and Bartlett, points out that Canada field pea seedlings made a fairly healthy growth in distilled water despite the loss of considerable quantities of electrolytes to the outer solution.

Hibbard (**10**) found that a slow process of adjustment or acclimatization to distilled water fitted *Lupinus alba* seedlings to better growth than

thrusting them directly into it. Finding that root growth was increased when the distilled water was changed 4 times daily, he concluded that the roots were injured because they secreted a substance toxic to themselves. SCARTH (17) found that boiling distilled water in silica vessels to expel carbon dioxide changed the pH from 5.5 to 7.1 and reduced the toxicity to Spirogyra. The tests were made in covered beakers but mention is not made that air or light was thereby excluded and the pH of the distilled water after the algae had been in it for some hours is not given.

The water used in the present experiments was from a gas-heated BARNSTEAD still and it was stored in tin-lined copper tanks. It was not redistilled and it is not known to what extent the carbon black on the roots adsorbed traces of copper or other metals.

As shown by table VII the roots of both corn and tomato made a better

TABLE VII

CORN AND TOMATOES WITH ROOTS DIVIDED BETWEEN DISTILLED WATER AND 2 × BASE NUTRIENT. EXPERIMENTS 10, 11, AND 12

Container	Solution designation	New solutions		New roots		Total water uptake	
		Δ	K × 10⁻⁵ at 25° C.				
		°C.		gm.	%	ml.	%
Experiment 10. Two corn plants. Duration 34 days							
A	Distilled water		.45	4.3	11	588	12
B	2 × base nutrient	0.113	413.00	34.0	89	4126	88
Experiment 11. Two tomato plants. Duration 17 days. Stems not split							
A	Distilled water		.45	10.3	38	1175	42
B	2 × base nutrient	0.113	413.00	16.7	62	1605	58
Experiment 12. Two tomato plants. Duration 34 days. Stems split up about 5 cm.							
A	Distilled water		.45	3.8	9	2886	30
B	2 × base nutrient	0.113	413.00	39.0	91	6647	70

growth in the nutrient solution than in the distilled water and for the full duration of the experiment more water was taken up from the solution of higher concentration. During the first days of all three experiments, however, relatively more water was removed from the distilled water vessels (table VIII) than from 2 × base nutrient; thus indicating that the injury to the roots—whatever its cause or character—did not prevent what was apparently a normal uptake of water. Later, when new and actively grow-

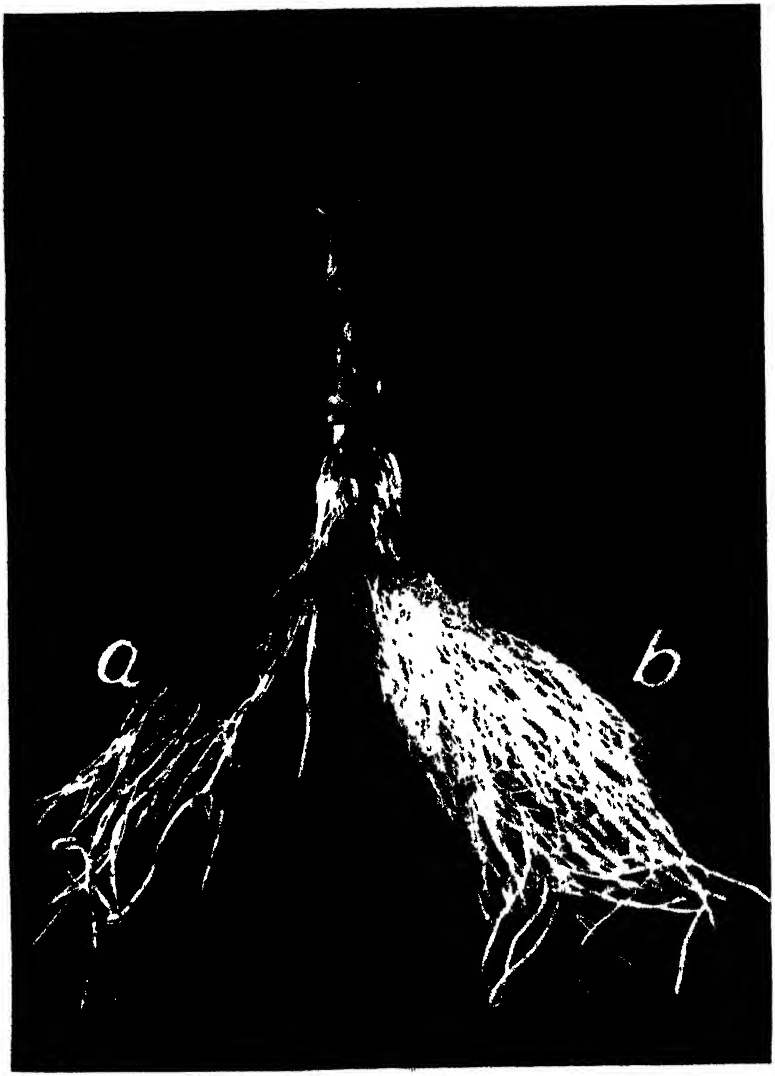

FIG. 3. Corn roots. a, in distilled water; b, in 2 × base nutrient.

ing roots had developed in the containers with nutrient solution, the rate of uptake from the nutrient solution exceeded that from the distilled water. The original roots constituted a greater proportion of final roots in these tomato experiments than in the corn experiment and relatively more water was taken up from the distilled water by the tomatoes than by corn (figs. 3 and 4).

There is substantial evidence (table VIII) in each of these three experi-

TABLE VIII

WATER UPTAKE BY PERIODS AND CONDUCTANCE OF USED SOLUTIONS AT END OF PERIODS. EXPERIMENTS 10, 11, AND 12

CONTAINER	SOLUTION DESIGNATION	WATER USE BY PERIODS				CONDUCTANCES OF SOLUTIONS REMOVED		
		0 to 3*	0 to 10	11 to 17	18 to 34	10th	17th	34th
		ml.	*ml.*	*ml.*	*ml.*			
		Experiment 10. Corn						
A	Distilled water	90	145	79	364	9.22	0.99	1.32
B	2 × base nutrient	40	167	470	3489	356.00	305.00	296.00†
		Experiment 11. Tomato						
A	Distilled water	190	590	585		6.64	0.96	
B	2 × base nutrient	140	750	855		225.00	202.00	
		Experiment 12. Tomato						
A	Distilled water	190	405	595	1886	10.70	1.56	6.85
B	2 × base nutrient	90	345	918	5384	350.00	205.00	232.90†

* Approximate since solutions were not removed from containers for measurement.
† This solution renewed on 31st day—conductance not recorded.

ments indicating the movement of solutes from the roots into the distilled water; the nature of the electrolyte so moved, however, was not determined. A conductance of 10×10^{-5}, such as was observed in the first period of both experiments 10 and 12, corresponds to about 1 m.e. of cations per liter.

Discussion and conclusions

WATER RELATIONS

Experiment 9 provides a suitable basis for discussion of the effect of solution concentrations on water uptake by divided root systems. It was found that 1.86 ml. of water was withdrawn from the nutrient solution with an osmotic pressure of 0.3 atm. for each 1 ml. of water withdrawn from the nutrient solution with an osmotic pressure of 1.8 atm. Inasmuch as the characteristics of the two halves of the root system remained equal throughout the test it may be possible to estimate the magnitude of a maintained tension in the water in the base of the shoot that would be necessary to satisfy both of the two pairs of values (table VI, fig. 5).

The forces peculiar to living cells and tissue are neglected in the calculations that follow and it becomes of interest for this reason to consider to what extent such omissions are justified. Guttation is believed in some

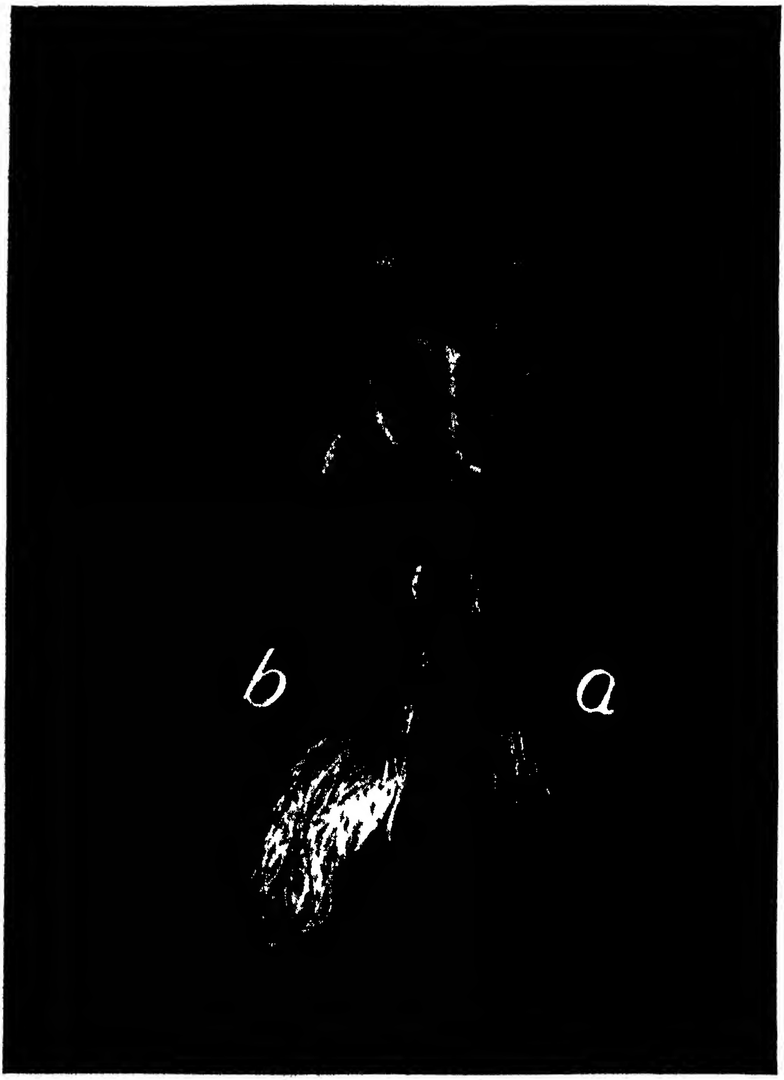

FIG. 4. Tomato roots. a, in distilled water; b, in 2 × base nutrient.

instances to represent glandular action; MASON and PHILLIS (13) have shown that the osmotic pressures of protoplasmic saps are greatly in excess of vacuolar saps. This suggests that forces originating through respiratory activity of protoplasm may be important in the secretion of dilute solutions; even possibly of those passing from the root cortex into the xylem. Evidence from experiments by KRAMER (12) and others, however, support the view that forces arising from metabolic activity of root cells that tend to

move water into and through roots is negligible or quite secondary to forces created as a result of transpiration. It is the writer's opinion that exudation from decapitated plants (and exudation does not always occur) represents in large measure water movement brought about by the osmotic pressure of the tracheal sap when it is in excess of the sum of the osmotic pressure of the soil solution and capillary forces of the soil. In an intact plant even this force is of doubtful significance, except possibly during the recovery of turgidity, since the osmotic pressures in the foliar cells are greater than those of tracheal saps. The movement of water to leaves during transpiration (and after transpiration before turgidity has again been established) can be looked upon most logically as being the result of osmotic forces in excess of the inwardly directed forces exerted by cell walls. Plant roots can not be likened either to cylindrical tubes or to a porous medium such as soil. It is still probable, however, that the movement of water through roots obeys the law that has been found to hold in these other materials.

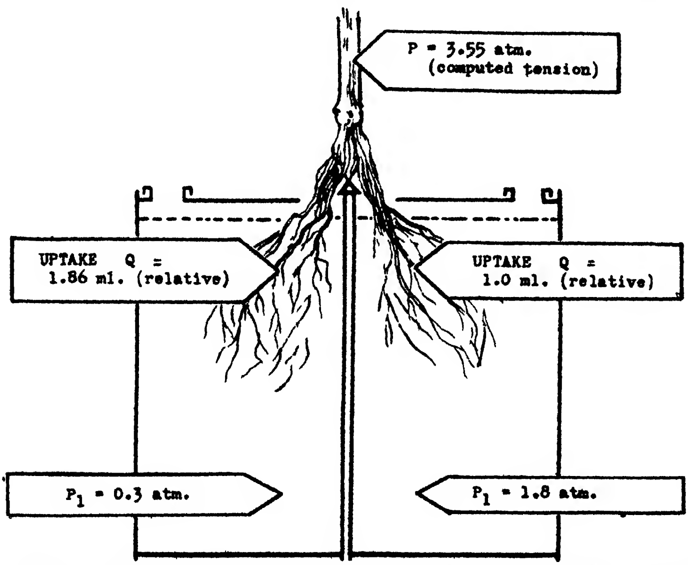

FIG. 5. Experiment 9. Water uptake, osmotic pressure, and tension relationships.

The flow of liquids through cylindrical tubes and through porous media has been found by POISEUILLE and DARCY, respectively, to be directly proportional to the difference in the hydraulic head of the liquid. For an approximate solution to the present problem, the most reasonable assumption we can make is that a similar relation holds for the transport of liquids through the conducting tissue of the plant. On the basis of this assumption and under present conditions where the gravity force is negligible compared with the pressure gradient force, one may write

$$Q = K(P - P_1)$$

where Q is the volume of flow in unit time, and $P - P_1$ is the difference in the pressure between the liquid in the conducting system and the liquid surrounding the roots. It is further assumed that osmotic and hydrostatic pressure differences are equally effective in producing the flow. The single value for the tension, P, of the water in the base of the shoot that satisfies both sets of water uptake and osmotic values is 3.55 atm. This value was obtained by substituting the two sets of experimental data in the above equation to obtain $1.86 = K (P - 0.3)$ and $1.0 = K (P - 1.8)$. Solving these equations simultaneously gives $P = 3.55$ atmospheres.

The foregoing value is necessarily an average one just as the water uptake values were the average of day and night uptake over the period of the experiment. With high transpiration rates the tension in the stem would become large, whereas at night as the plant regained full turgidity the tension would approach zero or become a positive hydrostatic pressure. From these relations it follows that as the tension increased the differences in the rate of uptake from the two solutions would narrow. On the foregoing basis, a tension of 10 atm. should produce an uptake of 1.15 ml. from the dilute solution for each 1 ml. uptake from the concentrated solution. As the tension fell to approach the value 1.8 atm., water would be withdrawn only from the dilute solution. Theoretically, at yet lower tensions there should be a movement of water through the roots from the dilute to the concentrated solution. It was not determined if this was the case, but BREAZEALE (3) has reported such flow through roots from moist soils into dry soils. The tension on the moisture of soils in the wilting range is about 16 atm., and according to recent experimental data by L. A. RICHARDS the soil moisture tension at the moisture equivalent is about 0.5 atmospheres. It is of some interest to note at this point that KEARNEY (11) found salts to have no effect on the wilting coefficient. This implies that if salts in the soil solution affect the tension between soil and plant there is some compensating effect within the plant.

It has been repeatedly found that the water requirements of plants on single solutions high in nutrient, chloride, or sulphate salts tend to be substantially lower than those of control plants. It has seemed more reasonable to attribute this to reduced transpiration rates resulting from salt effects within the leaves than to reductions in the osmotic differentials between the leaves and substrate.

ROOT DEVELOPMENT

The greater development of roots in the dilute than in the concentrated solutions (distilled water experiments as exceptions) might be attributed: (1), to conditions unfavorable to root growth in the concentrated solutions; (2), to conditions particularly favorable to root growth in the dilute solutions; or (3), to factors within the roots that caused more of the organic

solutes translocated downward through the stem either to move into the dilute-solution roots or, having moved in, to be elaborated into tissue more rapidly. Either of these latter explanations would imply that the total growth of the root system as a whole had not been affected.

The fact that chloride, sulphate, and concentrated nutrient salts had similar effects would tend to discount the view that the concentrated solutions were especially unfavorable for root growth if all of the roots had been in one or the other kind of solution. It was noted in connection with experiment 1, where there was only a negligible root growth in the solution with 50 m.e. of chloride, that in other experiments with corn the roots of plants with all roots in 50 m.e. of chloride constituted a greater proportion of the weight of entire plants than was the case with plants growing with all roots in control solutions. In experiment 3 no appreciable difference was found in the moisture content of roots in the dilute and concentrated solutions.

In the absence of measurements on the distribution and concentrations of carbohydrates and salts in the two sets of roots of any experiment, discussion cannot be extended to the pertinent question of whether salt accumulations in the root tissues in the concentrated solutions tended to deflect the movement of organic materials from the shoots into the roots growing in the dilute solutions. A reason for a more rapid elaboration of synthesized products into cell structure in the roots containing less salt is not evident but the possibility that such was the case remains. A more rapid utilization of these materials by the dilute-solution roots would tend to establish more favorable translocation gradients to them.

As a final consideration some attention may be given to relations between the turgor pressures of roots and root growth. Cell growth by enlargement must be related to turgor pressure but one might question the extent to which the laying down of wall materials would be similarly influenced. ROBERTS (**16**) found the concentration of the cell sap of root hairs of radish growth in a series of 0.02 M to 0.65 M sucrose solutions to increase with the concentration of the external medium and maintain osmotic pressures 4 to 6 atm. above it. As further evidence of the adjustment of the plant to its substrate EATON (**6**) has presented the results of cryoscopic measurements on the expressed sap of a series of crop plants grown on saline solutions. The expressed sap of leaves of tomatoes, from the same seed stock as the present plants, when grown in out-of-doors sand cultures maintained osmotic differentials of 9.4, 8.9, 8.8, 9.5, 9.3, and 9.3 atm., respectively, above nutrient solutions as follows: control, plus 50 and 150 m.e. chloride, and plus 50, 150, and 250 m.e. sulphate. With this indication that the osmotic differentials between the root cells or leaves of a plant and its substrate are not greatly changed, within reasonable limits, by solutes added to the substrate, any conclusion with respect to disadvantages in water relations of the saline roots would have to be arrived at on some other basis. Otherwise,

one might reason that since accumulation ratios are relatively high when plants are on dilute media and low when on concentrated media, that higher turgor pressures would exist in the dilute-solution roots than in the concentrated-solution roots. In other words, whatever the cause of the lesser root growth in the concentrated solutions may be, it is not reasonable to assign the cause to lack of turgor in the cells of the concentrated-solution roots.

Agronomic aspects

Prominent among the factors that tend to create differences in the concentration of the soil solution from point to point in irrigated soils there are especially to be mentioned: (1), variations in the permeability of proximately situated soil zones, even though of similar texture; and (2), the usually greater uptake by the plant of water than of salt, thereby increasing the concentration of the soil solution. The first-mentioned consideration gives rise to much lateral diversity since the less permeable zones are not leached as effectively as the permeable zones. The second consideration is largely responsible for the pronounced tendency for salt concentrations to increase with depth. The portion of a soil solution that is residual from a particular irrigation tends to be displaced downward in successive steps by successive irrigations and rains; thus the unused fraction ultimately lies deep in the root zone with a greatly reduced volume but still containing much of its initial salt.

Among other factors that influence both the average concentration of the soil solution as well as variations within the root zone, there are to be mentioned such considerations as the quality of the irrigation water, the extent to which water is used in excess of crop needs to promote leaching of the salts from the root zone, the amount of rainfall, methods of applying water to the land, and subsoil drainage conditions. Salt impregnated layers of clay as well as saline water tables are sometimes the source of salt brought into the upper root zone with rising moisture.

Both lateral and vertical diversity were illustrated in table I. As features pertinent to that example, it is to be noted that it had been the owner's policy to apply only sufficient irrigation water to maintain the trees from one irrigation to the next. Since the rainfall over a period of years prior to the collection of those samples had been subnormal there had been little recent leaching of the lower root zone. After two winters with leaching rains a much more uniform salt distribution was found. The irrigation water was of good quality containing but 0.29 m.e. of sulphate and 0.48 m.e. of chloride per liter. The soil samples were collected from beneath the irrigation furrows nearest the trees.

Summary

Corn and tomato plants were grown with their roots divided between two or more solutions of unequal concentration. They developed more

roots in the dilute than in the concentrated solutions and the water uptake from the former exceeded that from the latter. The foregoing was found irrespective of whether the differences in concentration were affected by the addition to the base nutrient of chloride, sulphate, or additional nutrient salts. Little difference in water uptake or in root growth resulted in solutions of similar osmotic pressure when one was high in chloride and the other in complete nutrient salts. These findings indicate that osmotic pressures, rather than specific ion effects, are primarily involved.

With an equal growth of the two halves a divided root system accomplished by alternating their positions each second day between dilute and concentrated nutrient solutions (0.3 and 1.8 atm.), 1.86 ml. of water was taken up from the dilute solution for each 1 ml. of water from the concentrated solution. A value of 3.55 atm. for the tension in the water of the stalk was found by calculation to satisfy these two sets of values on the basis of a first assumption that the observed water uptake by the two halves of this root system should be proportional to the difference in the osmotic pressure in the solutions and the tension in the water in the stem. This value is of course an average one and the data are discussed accordingly.

In an experiment parallel with the foregoing one, the two portions of the root system remained throughout in the solutions in which they were started. The new roots developed in the dilute solution weighed 1.6 times as much as those in concentrated solution and 76 per cent. of the total water taken up by the plant was from the dilute solution.

Roots divided between distilled water and concentrated nutrient made more growth in the latter. Almost twice as much water was withdrawn from the distilled water vessels during the first three days of the experiment as from the nutrient solution but this was later reversed.

The investigations were undertaken as a means of appraising the effects of the variability in the concentration of the soil solution often found within the root zone of plants growing on irrigated lands. When soils are permeable and extensively leached this variability may be slight but under other circumstances it may be great. An example is presented of the situation found in a permeable but lightly irrigated orchard soil wherein 15- to 60-fold variations in the concentrations of sulphate and chloride, respectively, were found within the root zone of single trees.

The writer acknowledges with appreciation the assistance of G. Y. Blair in this investigation.

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INFLUENCE OF SOIL MOISTURE ON PHOTOSYNTHESIS, RESPIRATION, AND TRANSPIRATION OF APPLE LEAVES[1]

G. WILLIAM SCHNEIDER AND N. F. CHILDERS

(WITH THREE FIGURES)

Introduction

A deficiency of water under natural and even cultural conditions is probably responsible for the poor growth and death of more plants than diseases, insects, or any other cause. Although it is possible to see with the naked eye how and when a plant responds to low water supply, it is of interest to know the extent to which a drying soil affects metabolic processes before and during the time wilting is visible. It is of interest also to know whether a wilted plant recovers immediately in leaf activity when water has been supplied to the soil, or if normal activity is delayed or never again attained. The results presented in this paper trace the daily effect of a gradually drying soil on apparent photosynthesis, respiration, and transpiration of small apple trees as the soil in which they were growing gradually dried to approximately the wilting percentage, after which it was watered to field capacity.

Methods

ENVIRONMENT-CONTROL CHAMBER EXPERIMENTS

In the Horticulture and Forestry Building, Ohio State University, a large insulated chamber (**6**) seven by eight by eight feet high, inside dimensions, has been constructed for the purpose of controlling light, temperature, and relative humidity while leaf metabolism tests are being made. At the time of these experiments no effort was made to control the amount of carbon dioxide in the air. The air replacing that removed by the pump was drawn from the outside of the building. Carbon dioxide in the air usually averaged about 15 to 17 milligrams per cubic foot, corrected to standard temperature and pressure.

The apparatus used and the procedure followed to determine the carbon dioxide exchange was similar to that previously described by HEINICKE (**9**) and HEINICKE and HOFFMAN (**11**) except that a mercury piston air pump[2] was used to draw the air over the leaves. This method measures the amount of carbon dioxide absorbed by the leaf and is therefore a measure of *apparent* photosynthesis and not *actual* photosynthesis. Thus whenever the term

[1] This paper has been published in abstract elsewhere (7).

[2] Thanks are expressed to DR. A. J. HEINICKE, Cornell University, who devised the pump and granted us special permission to use it in our experiments.

photosynthesis is used in this paper it should be considered as meaning apparent photosynthesis.

The Stayman and McIntosh apple trees used in the chamber experiments were two years old and growing in five- and ten-gallon glazed stone crocks. The soil in these containers had a moisture equivalent of 36.4 per cent. and a wilting percentage of 15. 2 (determined by analyzing the soil after dwarf sunflowers had grown in it to about 10 inches in height, then allowed to wilt). The trees had been cut back to 8-inch stubs when set in the greenhouse in December, 1938, and one vigorous shoot was allowed to grow from each stub. Six leaves were selected for study on the check tree and six on the test tree, all of which were evenly spaced around the three- to four-foot shoots. Terminal buds did not form on any of the test trees during periods of experimentation.

Daily determinations started at 11:00 A.M. and were run for three hours, immediately after which several respiration determinations were made at times specified later. To make a respiration determination the lights were turned off and an office heating cone, which had been attached to an oscillating fan, was turned on to help maintain with the cooling system a constant temperature level.

The entire bank of lights was usually turned on about 7:45 A.M. each morning. They were turned off between 5:00 and 6:00 P.M. since earlier tests have indicated that small apple trees should receive equal amounts of light each day if a fairly consistent relationship in leaf activity from one day to the next is maintained between two trees.

Transpiration was measured by a method similar to one described previously (**10**). The system consisted of several Freshman bottles (2 inches in diameter by 4½ inches high, one for each leaf and air-check line) which contained ground pumice stone impregnated with sulphuric acid. The acid efficiently removed water vapor from air flowing through 9-mm. glass tubing at the rate of three to four cubic feet per hour, and did not interfere with the carbon dioxide to be removed later in the absorption towers. After each determination the rubber stoppers, with glass attachments and metal numbers, were removed from the bottles which, in turn, were placed in a drying oven at about 160° C. After 24 hours the bottles were removed and allowed to cool to air temperature, stoppered, and held ready for the next run. These bottles could be used for a month or more until the absorption medium became somewhat weak and it was necessary to impregnate the stones again with sulphuric acid. The common laboratory procedure for preparing these stones was essentially as follows: Place pumice stones about the size of peas in an evaporating dish or casserole and cover with concentrated sulphuric acid. Place on a hot plate and heat until dense white fumes appear, then continue the heating for one-half hour. Allow to cool and drain off all of

the acid. Again heat the pumice until heavy white fumes of sulphur trioxide are given off. At the end of this heating, there should be no excess of sulphuric acid present. Cool in a desiccator and store in stoppered glass containers.

General procedure

The general procedure employed for the water deficiency experiments was essentially as follows: Determinations of apparent photosynthesis, transpiration and, in some cases, respiration were made from three to five days before the dry period began in order to establish a relationship in leaf activity between the group of leaves to be used as test leaves and those to remain as controls. When a consistent relationship had been established, the test tree ceased to receive water while the check, or control, tree received it daily in order to keep the soil at approximately the field capacity. Daily determinations were continued until the test plant had reached the incipient or permanent wilting stage (as specified later) when the soil was again watered to field capacity and the runs continued for 7 to 10 days. Stomatal observations were made either by removing small sections one centimeter square from neighboring leaves on the shoot and immediately inspecting them under the microscope, or by observing the inverted intact leaf through a mounted microscope.

Field experiments

The assembly of carbon dioxide absorption towers, electric air pump (instead of mercury piston pump), and wet test meter were located in the greenhouse and connected by glass tubing to individual leaves on two Stayman apple trees. The trees were growing in five-gallon glazed stone crocks outside the greenhouse where they were fully exposed to the sun. Soil surrounding their root systems had a moisture equivalent of 38.4 per cent. and a wilting percentage of 15.55. Leaf cups were attached to six leaves on the test plant and to four leaves on the check while two of the air lines were reserved to determine the amount of carbon dioxide in air before it reached the leaves. Leaves selected on each tree were evenly distributed up and down and around the four- to five-foot shoots. Two four-hour determinations were usually made each day, the first beginning about 7:00 A.M. and the second about 11:30 A.M. Light and temperature readings were recorded at the beginning, middle, and end of each run with a Weston illuminometer and a Fahrenheit dairy thermometer.

Results

Environment-control chamber

Four experiments were performed in the environment-control chamber. In the first test only apparent photosynthesis determinations were made; in

the second and third experiments apparent photosynthesis, respiration, and transpiration data were recorded; in the fourth only respiration was studied.

EXPERIMENT I (APPARENT PHOTOSYNTHESIS).—The trees used in this experiment were of the McIntosh variety; tree A was the check and tree B the test plant. During each determination the temperature was held at 80° F., and light intensity was constant at an average of about 4000 foot candles for all leaves concerned. Relative humidity was approximately 30 per cent. during the light period. A relationship between the photosynthetic activity of trees A and B was established in the eight-day period from February 14 to February 21. The data in table I show the daily rate of apparent photosynthesis in milligrams of carbon dioxide absorbed per 100 square centimeters of leaf surface per hour, before and after the dry period. The test tree, B, received its last watering the night of February 20, while the check tree, A, received it daily throughout the experiment. The leaf relationship during the standardization period showed that for every 100 milligrams of carbon dioxide assimilated by the check plant the test plant utilized 128.9 milligrams. Thus, the *expected rate* of the test plant was 128.9 per cent. of the check (see foot-note in table I for explanation of figures termed *expected rate and percentage expected rate*). During the first four days of the dry period from February 22 to 25, the test plant showed an average increase in carbon dioxide assimilation of about three per cent. above its rate previous to the treatment. From February 25 until March 3 there was a gradual decrease in the rate of photosynthesis of tree B from 101 to 76 per cent. of the expected rate. At no time during this period did the test tree show signs of wilting, even by late afternoon when the lights were usually turned off. On March 4 there was a sharp reduction in photosynthesis, to 46 per cent. of the expected rate; no wilting was evident, however, until about three hours following the determination when the leaves appeared to be slightly flaccid. The next day, March 5, the test tree was fairly turgid at the start of the run but was wilted at the end of the determination, with the top leaves showing the greatest effect of water deficiency. The entire tree showed signs of wilting on the following morning of March 6 when the lights were turned on. The soil continued drying until March 11 when assimilation had gradually fallen to around 14 per cent. of the expected rate. Stomata on the test tree appeared to be closed continuously during the period of wilting, including the mornings before determinations were started. It is interesting to note that fairly good values for photosynthesis were obtained when the leaves were somewhat wilted and the stomata appeared to be closed. On March 11, about two hours before the determination, water was added to the dry plant and by starting time the tree had almost recovered to complete turgidity. Within another hour the stomata were almost as far open as were those on the check leaves (75 to 100 per cent.).

TABLE I

EFFECT OF SOIL MOISTURE ON THE RATE OF PHOTOSYNTHESIS OF McINTOSH APPLE LEAVES. EXPERIMENT I, TEMPERATURE 80° F.

DATE, 1939	APPARENT VALUES OF PHOTOSYNTHESIS IN CO_2/100 CM^2/HR.		EXPECTED RATE OF TEST TREE
	CONTROL	TEST	
	mg.	*mg.*	%
Feb. 14	31.4	37.7	......
15	26.8	29.6	......
16	21.7	30.3	......
17	23.0	33.0	......
18	12.9	15.2	......
19	17.6	26.1	......
20*	15.4	24.4	......
21	19.7	21.6	......
Average	21.1	27.2	128.9‡
22	11.2	15.0	103.9§
23	15.2	20.1	102.6
24	11.8	16.0	105.1
25	12.5	16.3	101.2
26	18.9	19.1	78.4
27	14.0	11.1	61.4
Mar. 1	7.6	7.5	76.5
2	12.4	12.2	76.3
3	15.7	15.4	76.1
4	8.7	5.0**	44.6
5	20.8	15.4	49.7
6	13.6	5.4	30.8
7	9.1	5.4	46.0
8	15.9	2.3	11.2
9	9.2	2.2	18.5
10	12.4	3.2	13.8
11†	10.3	8.8	66.3
12	10.3	8.5	64.0
13	12.2	16.5	104.9
16	10.0	14.9	115.6
17	10.5	13.7	101.2
18	16.0	20.4	98.9
19	11.1	14.9	104.1
20	8.6	10.9	98.3
21	10.5	15.5	114.5
22	15.9	17.9	87.4
23	7.9	8.6	84.5
24	11.9	13.8	90.0
25	12.6	14.2	87.4

* The test plant last received water on the evening of February 20.

† Test plant watered to field capacity of the soil two hours before determination was made.

‡ Obtained by dividing the average apparent photosynthetic value (27.2) for the test tree by the average value (21.1) for the check tree and multiplying by 100.

§ Obtained by dividing the daily rate of photosynthesis of the test tree (15.0) by the daily rate of photosynthesis of the check plant (11.2), multiplying by 100 and dividing the product by 128.9.

** Test tree showed first signs wilting two hours after run.

Assimilation during this run recovered to 66 per cent. of its expected rate and within 48 hours had returned to its original status.

From March 11 to 15 the test tree was watered daily and then allowed to dry again. During the six-day period which followed March 15, apparent photosynthesis of the test plant averaged 105.4 per cent. of its expected rate; or, in other words, it showed a 5 per cent. increase which corresponded with the average 3 per cent. increase obtained at the beginning of the first dry period from February 22 to 25. Beginning with March 22, however, photosynthetic activity began to decrease at a rate similar to the first test. The experiment was discontinued.

EXPERIMENT II (APPARENT PHOTOSYNTHESIS, RESPIRATION, AND TRANSPIRATION).—Stayman Winesap trees were used in this experiment. Temperature in the chamber during each determination was constant at 90° F. and

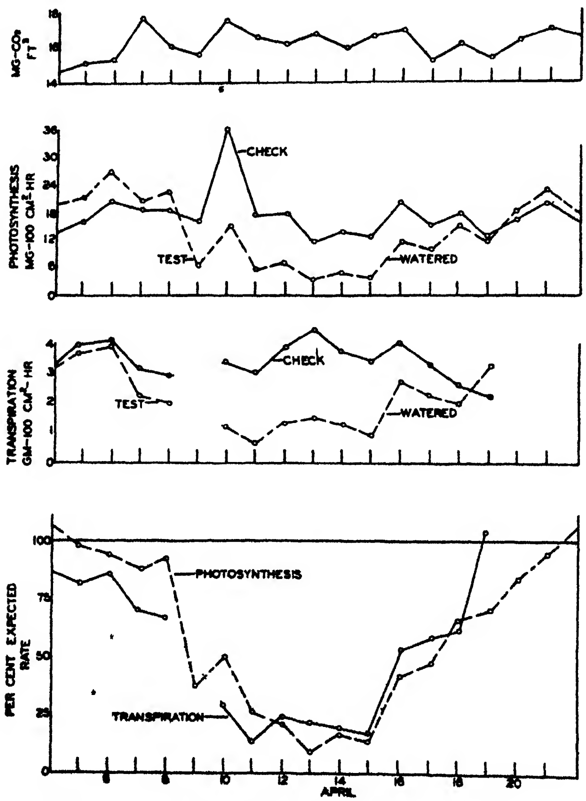

FIG. 1. The influence of a gradually drying soil on apparent photosynthesis and transpiration of apple leaves. The test tree was last given water the evening of April 1. First wilting occurred the afternoon of April 10; soil was watered to field capacity two hours before the determination on April 16 (experiment II).

light averaged approximately 4000 foot candles for the six test and six check leaves. These factors did not vary during the nine-hour day from 8:00 A.M. to 5:00 P.M. At other hours of the day the lights were turned off and the plants remained at room temperature which was about 75° F.

Data for this experiment are presented in table II and graphically shown

TABLE II

INFLUENCE OF SOIL MOISTURE ON PHOTOSYNTHESIS, RESPIRATION, AND TRANSPIRATION OF STAYMAN APPLE LEAVES. EXPERIMENT II, TEMPERATURE 90° F.

DATE, 1939	APPARENT VALUES PER HOUR PER 100 CM^2						EXPECTED RATE OF TEST TREE*	
	ASSIMILATION CO_2		TRANSPIRATION H_2O		RESPIRATION CO_2			
	CONTROL	TEST	CONTROL	TEST	CONTROL	TEST	ASSIM.	TRANSP.
	mg.	*mg.*	*gm.*	*gm.*	*mg.*	*mg.*	%	%
Mar. 27	22.1	26.7	1.9	2.0				
28	14.1	20.1						
29	12.9	17.1	3.2	3.4				
30	22.6	30.7	2.9	3.7				
31	19.4	25.9	2.7	3.6				
Apr. 1†	17.8	22.8	3.5	3.7				
2	16.2	22.1	3.3	3.9				
Average	17.9	23.6	2.9	3.4			131.8	117.2
4	13.0	18.2	3.4	3.4	3.6	2.0	106.2	85.3
5	15.7	19.8	3.8	3.6			95.6	80.8
6	20.5	24.8	3.9	3.8	7.0	6.0	91.7	83.1
7	18.4	21.3	3.2	2.4			87.8	64.0
8	18.5	22.0	2.8	2.0			90.2	60.9
9	17.1	5.9					26.2	
10	36.4	17.6	3.3	1.0			36.6	25.9
11	18.6	4.2	2.9	0.5	2.6	4.6	17.1	14.7
12	18.6	5.4	3.5	1.0	5.5	6.5	22.0	24.4
13	11.9	1.4	4.2	1.0			8.9	20.3
14	13.8	2.5	3.3	0.6	1.5	3.5	13.7	15.5
15	12.6	2.2	3.2	0.5	4.7	5.2	13.2	13.3
16‡	19.9	11.7	3.8	2.3	3.3	4.0	44.5	51.6
17	14.7	9.5	3.1	1.8	3.8	4.8	49.0	58.0
18	16.5	14.7	2.5	1.6	4.2	5.0	67.6	54.6
19	13.0	12.7	2.4	2.9	2.9	2.9	74.1	103.1
20	14.6	16.6			3.6	3.4	86.3	
21	16.7	20.9			2.9	1.5	94.9	
22	10.5	14.7					106.2	

* See table I for method of calculation.

† Test tree last watered the evening of April 1. (No determination made first day of dry period, April 3.)

‡ Test tree thoroughly watered two hours before test period began.

in figure 1. The relationship between the control tree, C, and the test tree, D, was established in the seven-day period from March 27 until April 2. During this standardization period the test plant was found to have an expected photosynthetic rate of 131.8 per cent. and an expected rate of transpiration of 117.2 per cent. No determinations were made on April 3.

On April 4 the test tree showed an increase of 6 per cent. in photosynthesis. This increased rate of photosynthesis during the early part of the dry period apparently lasted at the most only two days. This shorter period of increased photosynthetic activity, as compared with the four-day period in the previous experiment, was probably due to the 10 degree higher temperature which caused a more rapid drying of the soil and a higher rate of transpiration by the leaves. It is interesting to note that while photosynthesis showed a six per cent. increase on April 4, transpiration showed a 15 per cent. decrease. On the following day, April 5, the percentage expected rate of apparent photosynthesis was about 4 per cent. below its rate previous to the test period; transpiration, however, showed about a 19 per cent. decrease. From April 5 until April 8 photosynthesis of the test tree showed a 19 per cent. decrease and transpiration a 40 per cent. decrease. It is apparent from these data that transpiration showed a marked drop two days before an appreciable reduction in photosynthesis occurred. The test tree showed slight wilting at the tip at the conclusion of the run on April 10. It had recovered, however, within two hours after the lights were turned off in the afternoon. In spite of the slight wilting which occurred at the end of this three-hour run the test plant assimilated carbon dioxide at the fairly good rate of 17.6 milligrams per 100 square centimeters of leaf surface per hour. On April 11 the lights were turned on about three hours before the run began, and at starting time the plant again showed signs of slight wilting at the tip. By termination of this run all leaves were drooping. Carbon dioxide assimilation was 17 per cent. of the expected rate while transpiration was only 15 per cent. of its expected rate. In the period from April 11 to 16 as the plant continued to dry, the photosynthetic activity and transpiration of tree C dropped in each case to about 13 per cent. when the soil in which it was growing had reached a definite wilting percentage.

About two hours before the run was started on April 16 the test tree was thoroughly watered but it did not recover to full turgidity until about three hours after the lights had been turned off in late afternoon. Although the plant was obviously wilted during this run, the rate of photosynthesis and transpiration increased to 44 and 52 per cent., respectively, of their original rate. By April 19 carbon dioxide assimilation of the test plant had increased to about 74 per cent. of its former rate, while transpiration showed a rapid increase to 106 per cent. On the day following this increase in transpiration, photosynthetic activity increased to 86 per cent., and by April 22 showed complete recovery to 106 per cent. of the expected rate. Thus, the time required for this plant to return to about normal transpiration and assimilation after the dry period was four and seven days, respectively.

A three-hour respiration determination was made on several occasions following the above daily tests. Conditions were maintained the same with

the exception that the chamber was darkened. Unfortunately no respiration runs were made before the start of the dry period. On April 4 and 6, however, respiration tests were made on the two sets of leaves when photosynthesis and transpiration had not as yet shown marked reductions. In table II it is apparent that the respiration rate of tree C, the test plant, was lower than that of the check tree, D, for both determinations. By April 11 when the next determination was made, however, respiration of the drying plant had increased above the check and this relationship continued during the dry period, and for four or five days after the water had been added on April 16. It is evident in table II that variations in the rate of respiration were directly opposite to variations in photosynthesis. When the former process showed an increase, the latter showed a decrease as the soil gradually dried; after water was added, the situation was slowly reversed.

EXPERIMENT III (APPARENT PHOTOSYNTHESIS, RESPIRATION, AND TRANSPIRATION).—This experiment was carried out under the same procedure and set of conditions as the previous experiment except the temperature was 10° F. higher (100° F.) and the test plant was allowed only to show incipient wilting before water was supplied. Tree E, the control, and tree F, the test were both of the McIntosh variety. During the standardization period, April 24 to April 28, both trees were watered daily in the evening until the evening of April 28 when only the control tree received water and the test plant was allowed to begin drying. In this experiment, as in the previous two experiments, shortly after the last watering the test plant showed an increase in photosynthetic activity over a two-day period, April 29 and 30, or an average increase of about seven per cent. Transpiration also showed a slight increase on but one day, April 29. According to the data in table III, it is evident again that transpiration shows a decrease before photosynthesis when water becomes limiting. Transpiration dropped slightly on May 30, and photosynthesis showed a slight decrease the next day; the first marked drop in transpiration came on May 3 while the first marked drop in photosynthesis occurred the following day. Toward the end of the determination on May 6 the test plant showed signs of wilting but recovered about two hours after the lights were turned off in the evening. Photosynthesis and transpiration on this day were 44 and 36 per cent., respectively, of their expected rate. Soil in which the test plant was growing was watered to field capacity late in the evening of May 6. The next day photosynthesis had recovered to almost its original status and had completely recovered by May 8. Transpiration showed complete recovery by May 9.

Immediately following each of the above determinations the chamber was darkened and respiration runs were made at the same temperature (100° F.) on four leaves on each of the above test and check plants (see

TABLE III

THE EFFECT OF SOIL MOISTURE ON PHOTOSYNTHESIS, TRANSPIRATION, AND RESPIRATION OF APPLE LEAVES. EXPERIMENT III, TEMPERATURE 100° F.

Date, 1939	Apparent values per hour per 100 cm²						Expected rate of the test trees*		
	Assimilation CO_2		Transpiration H_2O		Respiration CO_2				
	Control	Test	Control	Test	Control	Test	Assimilation	Transpiration	Respiration
	mg.	mg.	gm.	gm.	mg.	mg.	%	%	%
Apr. 24	14.6	12.2	2.5	2.7	....	......	... .	....	....
25	16.9	15.0	2.9	3.2	.. ..	. ...	. ... . .	....	....
26	20.6	19.5	3.0	3.2	6.3	4.1	....	....	....
27†	20.6	19.7	3.3	3.6	4.6	3.6	... .	. ...	.. ..
28	17.3	15.5	3.2	3.6	4.5	3.4	....	....	....
Average	18.0	16.4	3.0	3.3	5.1	3.7	91.1	110.0	72.5
29	21.5	20.5	2.9	3.3	.....	. . .	104.7	103.4	....
30	16.2	16.0	3.5	3.8	.	.. .	108.4	98.7	....
May 1	12.2	10.6	3.4	3.3	6.0	5.9	95.4	88.2	135.6
2	18.2	14.9	4.3	3.9	6.9	6.9	89.9	82.4	137.9
3	19.0	16.5	4.1	2.3	2.8	3.0	95.3	51.0	147.8
4	20.7	9.5	4.2	2.2	2.9	3.2	50.4	47.6	152.2
5	19.7	8.1	4.9	2.0	1.7	2.0	45.1	37.1	162.2
6‡	20.7	8.2	4.6	1.8	2.6	3.0	43.5	35.6	159.1
7	16.2	13.6	3.8	3.8	3.0	2.4	92.2	90.9	110.3
8	15.3	14.7	3.4	3.6		..	105.5	96.3	... .
9	12.5	11.8	3.2	3.7	4.0	2.8	103.6	105.0	96.6
10	18.8	17.4	..	.	.. .	.. ...	101.6	....	....

* See table I for method of calculation.
† Test tree last watered evening of April 27.
‡ Test tree watered thoroughly evening of May 6.

experiment IV regarding the remaining four leaf cups which were attached to another drying plant for respiration studies). The data are given in table III and shown graphically in figure 2. It is apparent again that as

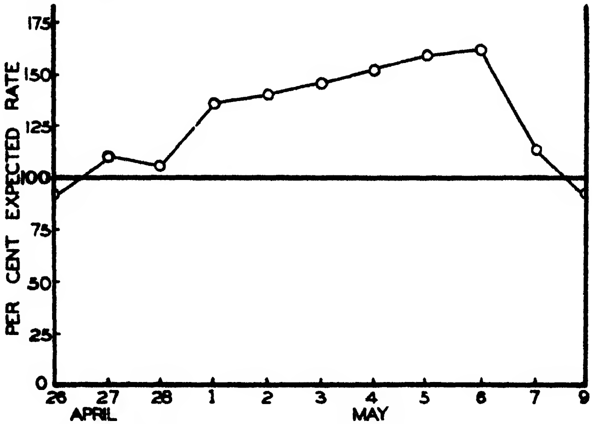

FIG. 2. The influence of soil moisture on respiration of apple leaves. The test tree received the last watering on the evening of April 27. It was watered again the evening of May 6 (experiment III).

water becomes limiting to an apple tree the respiration rate correspondingly increases, but when the plant has been watered again it gradually returns to its original relationship with the check plant. The highest rate of respiration, a 62 per cent. increase, was recorded on May 6 when transpiration and photosynthesis were lowest and the plant showed definite signs of wilting.

EXPERIMENT IV (RESPIRATION).—Data presented in table IV were collected on a McIntosh test tree, G, at the same time respiration determinations were made on tree F in experiment III. Leaf cups were attached to four leaves on test tree, G, while the control tree, E, remained as a reference in both experiments III and IV. The standard relationship between assimilation of the test and control leaves was established between May 4 and 6, and from the evening of May 6 to 9 inclusive the test tree received no additional water. In table IV it is evident that on the first day of the dry period, May 7, tree G showed the beginning of a gradual increase in respiration. On May 8, 9, and 10 there were further increases in respiration to 23, 34, and 30 per cent., respectively. After the test plant had been watered thoroughly the evening of May 10, it returned on May 11 to almost its original relationship in respiration with the reference tree. Thus, it is apparent from these data that when the dry period for an apple tree is short and less severe, respiration of its leaves returns from an increased rate to its normal status more quickly after water again has been supplied.

TABLE IV

EFFECT OF SOIL MOISTURE ON RESPIRATION OF MCINTOSH APPLE LEAVES. EXPERIMENT IV

DATE, 1939	APPARENT VALUES FOR RESPIRATION IN CO_2/ 100 CM^2/HR.		EXPECTED RATE OF THE TEST TREES*
	TEST	CHECK	
	mg.	*mg.*	%
May 4	2.9	4.5	....
5†	1.7	2.7	....
6	2.6	4.2	....
Average	2.4	3.8	158.3
7	3.0	5.7	120.0
8	2.2	4.3	123.4
9	4.0	8.5	134.2
10‡	1.7	3.5	130.0
11	2.2	3.7	106.3

* See table I for method of calculation.
† Test tree received last watering evening of May 5.
‡ Test tree watered to field capacity evening of May 10.

FIELD EXPERIMENTS[3]

During the summer of 1938 three experiments were carried out in a manner similar to those in the control chamber with the exception that the trees were placed outside the greenhouse where they were exposed to environmental conditions typical of central Ohio. The data are presented in figure 3 for one experiment which we considered, in general, almost identical with results obtained in the other two tests. Before it was evident that small variations in soil moisture had definite effects on leaf metabolism (as has been pointed out in the chamber experiments), it was decided to water the control plants in these tests only at intervals of 2 to 4 days when the soil appeared dry on the surface. The interval of watering obviously depended upon whether days were cloudy or sunny. This probably accounts for the fact that the relationship in photosynthesis between the two trees held practically constant for about three days after the dry period began (July 12) when the control tree also did not receive water for two days. Daily records show that water was applied to the control tree the mornings of July 12, 14, and the late afternoon of July 15 during the early part of the test period. From the afternoon of July 14 to the morning of July 18 the drying plant showed a definite relative increase (as much as 70 per cent., July 15) in carbon dioxide assimilation, which is similar to the behavior of drying plants in the chamber experiments. Beginning with the morning of July 19 there

[3] The authors appreciate the assistance of Mr. VERNON PATTERSON, senior in pomology, in performing the routine chemical analyses of the solutions.

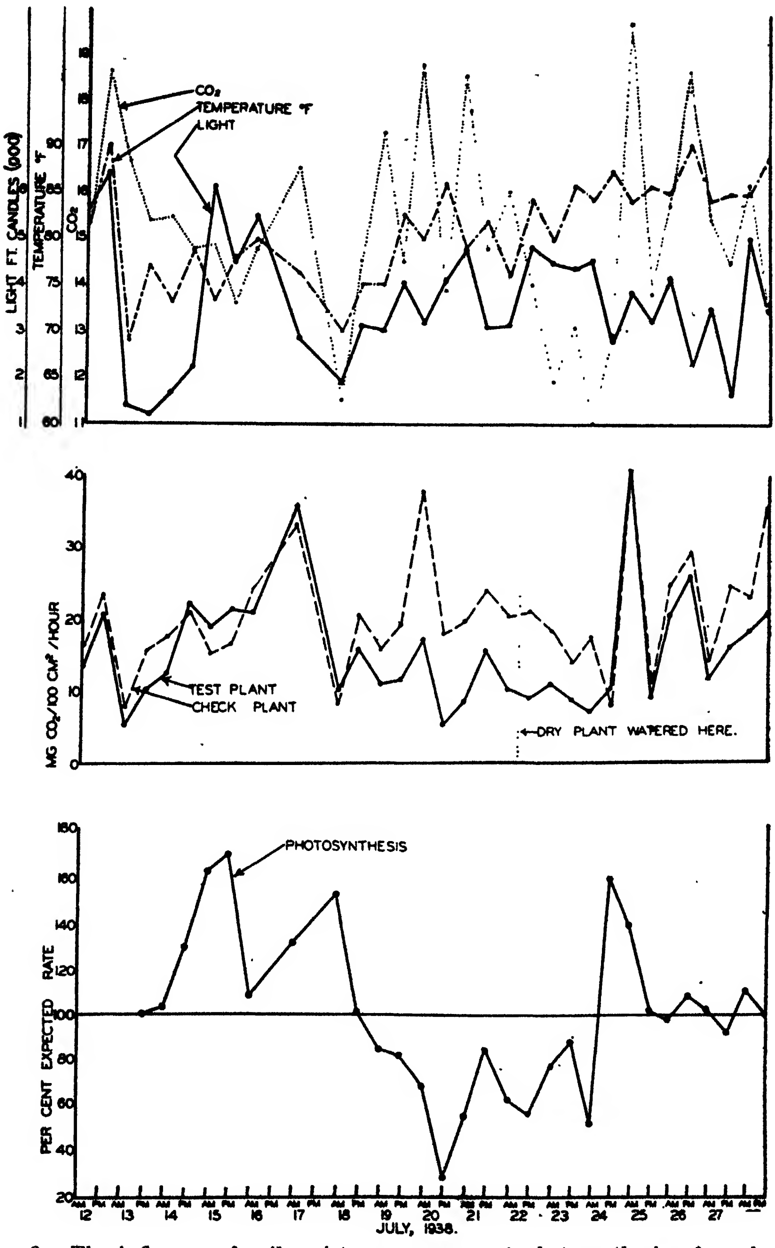

FIG. 3. The influence of soil moisture on apparent photosynthesis of apple leaves under central Ohio weather conditions in July. The test plant received water on July 12 and again on July 22 at 10:50 A.M. In the lower part of the figure the relationship in photosynthesis between the test and check trees on July 12 and 13 was taken as a standard for comparison of leaf activity on the remaining days.

was a gradual decrease in photosynthesis as the plant continued drying. During this period the weather was generally cool and often cloudy which afforded ideal conditions for a very gradual drying of the plant, thus avoiding any sudden scorching of the leaf tissues. The leaves on the test tree showed no signs of wilting until late afternoon of July 19 when slight wilting on the lower leaves occurred; this again stresses the fact that there may be a marked reduction in photosynthesis by apple leaves before wilting is visible to the eye. On the morning of July 20 the test tree had recovered, but by mid-afternoon two lower leaves were scorched at the tip and margins and the lower leaves, in general, were badly wilted. Again, early in the morning of July 21, the plant had shown noticeable recovery overnight, but by late afternoon several more leaves were scorched and the entire plant was very definitely wilted. At 10:50 A.M., July 22 the dry plant was thoroughly watered but did not recover until late afternoon. The morning of July 23 it appeared to be fully turgid, and according to figure 3 had recovered in photosynthesis in about three days. It then was allowed to begin drying again when the experiment was discontinued.

Discussion

The data in experiments I, II, and III obtained in the environment-control chamber and the data obtained in the field (fig. 3) have shown a consistent increase in photosynthesis when the moisture in a relatively heavy type of soil fell below its field capacity. In an earlier report by Heinicke and Childers (**11**) similar results were noted and it was suggested that "the optimum soil moisture for photosynthesis of apple trees may be below the field capacity of the soil, or certainly, below a degree of saturation which persists for a day or so after watering." This would seem reasonable to expect in view of the numerous reports (**2, 15, 21**) which stress the importance of good soil aeration as it influences root and top growth. From the standpoint of moisture content of the leaves, Brilliant (**4**) has shown that photosynthesis is at its maximum when leaf moisture was reduced 5 to 15 per cent. in *Hedera helix* and *Impatiens parviflora*. It may also be noted in experiment II (90° F.) that transpiration showed a decrease of 15 per cent. at a time when there was a 6 per cent. increase in photosynthesis. It is probable that if transpiration tests had been made at the lower temperature of 80° F. (experiment I) when the period of increase in photosynthesis extended over a four-day period, the above relationship between photosynthesis and transpiration would have been more pronounced.

It has been stated by Miller (**18**) that water plays both a direct and indirect rôle in photosynthesis: directly, by entering into chemical combination with carbon dioxide to form carbohydrates; and indirectly, by governing the opening of the stomata which, in turn, governs the intake of carbon

dioxide. In case of moderate water shortage to apple leaves, however, MAGNESS (**17**) states that there is so much water present in the leaf, even at time of stomatal closing, that it seems doubtful if a deficiency of water is a direct factor in reduced photosynthesis. In our experiments it has been difficult to establish a definite relationship between photosynthesis and stomatal opening at the time water becomes limiting; in several observations made by the junior author at Ithaca, N. Y., it appeared that there was a drop in carbon dioxide assimilation about the same time, or shortly after closing of the stomata was evident, due to water shortage. It does seem fairly clear, however, that under moderate weather conditions, there may be a 5 to 15 per cent. reduction in transpiration before stomata begin to show the effects of low water supply and before photosynthesis begins to show a definite decline (experiment II). MAGNESS (**17**) also found that moisture supply in the apple leaf may be reduced to an appreciable extent before stomata begin closing, "in some tests being approximately 5 to 7 per cent. higher at the time of stomatal opening than at the time of stomatal closing." He further states that under orchard conditions the early closing of stomata has proved to be about the best indication of the beginning of water shortage. Stomata, in most cases, were more sensitive to water deficiency than was the growth rate of the fruit.

With the type of soil used here (moisture equivalent of 38.4 per cent.; wilting coefficient of 15.55 per cent.) it is clear that there may be marked reductions in photosynthesis and transpiration and an increase in respiration before wilting in the apple is apparent. The length of time before wilting that these reductions may take place is dependent obviously upon the evaporating power of the air (experiments I (80°), II (90°), and III (100° F.)) and the mass of soil about the roots as well as the soil structure. The results presented here are in accord with those of LEWIS, WORK, and ALDRICH (**16**) who report in the case of heavy soils at Medford, Oregon, that fruit and tree growth were reduced significantly before soil about the majority of the roots of pear trees reached the wilting point. They state that fruit growth was reduced whenever soil moisture content fell below 70 per cent. of the available capacity. In case of the lighter soils (wilting coefficient of 8 to 10 per cent.) in California, however, HENDRICKSON and VEIHMEYER (**12**) have pointed out that fruit and tree growth were not reduced if the soil moisture remained about one per cent. or more above the wilting coefficient. HOWLETT (**13**) also states that with the Wooster Silt Loam (wilting coefficient of 7.5 to 8.0 per cent.) he was unable to detect any significant reduction in rate of fruit growth in the apple as long as the moisture was above the wilting coefficient.

It is generally considered uncertain that stomata are tightly closed even though they appear so under the microscope; it is interesting, however, to

note that on several occasions in these experiments we have obtained fairly high rates of photosynthesis when the stomata appeared to be completely closed. In his work with corn, SAYRE (19) has pointed out from considerable experience in the field where the "ultrapak" microscope was employed that "there is no close relation between stomata and photosynthesis in corn and that maximum photosynthesis can go on in the absence of open stomata." BERNBECK (1) also found that stomatal opening and photosynthesis operate independently under conditions of moderate water deficiency.

The time required for a wilted plant to recover to normal leaf activity after it has been watered is correlated, as one would expect, with the severity and length of the drought period [experiments II and III, and in the field (fig. 3)]. In experiment II, when the test plant was allowed to remain wilted for several days before watering, transpiration returned to approximately normal within 4 days while respiration and photosynthesis did not show complete recovery until the sixth and seventh day. In experiment III, where the plant was allowed to wilt only a single day, photosynthesis, transpiration, and respiration recovered within a day or two after the watering. Test plants in the field (fig. 3) were allowed to remain wilted for about two days and until several of the lower leaves on the shoots showed scorching and abscission before they were supplied with water. The time required for photosynthesis to recover was about two days. It should be pointed out that these reductions in leaf activity which persisted after water was supplied to the wilted plants, occurred even though the plants regained full turgidity within a few hours after watering. ILJIN (14) who worked with *Bidens tripartita* and *Phlomis pungens* also found that photosynthesis did not recover as soon as the wilted plants regained full turgidity but continued to show reductions of 29 per cent. 16 hours after recovery of turgor.

It is the opinion of YUNCKER (22) and others that when water supply to plants is reduced, the rate of respiration is likewise reduced; this does not seem to be the case in results presented here. In every case where respiration determinations were made on drying plants there was a relatively greater amount of carbon dioxide evolved from the drying leaves until the plant was watered, after which respiration gradually recovered to its normal relationship with the control tree. This is in line with the work of SMITH (20) who removed water from the leaves of snowdrop, stem tip of Tropaeolum, and young stems of Asparagus by means of a vacuum desiccator and showed that there was an increase in respiration proportional to the amount of water lost until 30 per cent. of the water had been removed. Respiration remained about the same when water was further diminished 50 to 60 per cent. From the data in tables II, III, and IV it is evident, also, that respiration of apple leaves assumed a more or less level status after the first two- or three-day climb, then dropped back upon addition of water.

In figures 1 and 3 it is evident that when data from the field are expressed in terms of percentage, the trend in photosynthesis is not so smoothly plotted as for data obtained in the environment-control chamber. For example, in figure 3, one would logically expect a greater reduction in assimilation of the drying plant on July 21 than on the previous day, July 20, because the soil, if anything, would be drier on the second day. The reverse, however, was true: there was a 66 per cent. reduction in photosynthesis on July 20 and only a 16 per cent. reduction on July 21. This and other unexpected variations in the photosynthetic relationship between the control and the test trees in the field might be explained by the wide variations in light, temperature, and other uncontrollable factors.

Summary

1. Determinations of apparent photosynthesis, transpiration and, in some cases, respiration were made on small apple trees while the soil in which they were growing gradually dried to the wilting percentage, after which it was watered to field capacity. The reference trees were watered at regular intervals. Experiments were performed both in the field and in a large insulated chamber where light was held constant at an average of about 4000 foot candles for all leaves concerned, humidity at about 30 per cent., and temperature at levels of 80°, 90° or 100° Fahrenheit.

2. The data show that an increase in apparent photosynthesis was associated with a slight decrease in soil moisture below the field capacity of the dark, fairly heavy soil employed in these experiments. Under temperature conditions of 100° F. this period of increase in photosynthesis was shorter (1 to 2 days) than when the temperature was 80° F. (2 to 4 days); this was probably due to the more rapid drying of the soil and the greater transpiration rate at the higher temperature.

3. At a temperature of 80° F. the first reduction in apparent photosynthesis occurred the fifth day after the last watering; at temperatures of 90° and 100° F. the first reduction in assimilation occurred on the third day. Ordinarily, under conditions of a gradually drying soil, there was a reduction in the rate of transpiration for at least one day before there was a reduction in photosynthesis. If evaporation conditions were low, this reduction in transpiration sometimes extended over a longer period of time before photosynthesis showed a definite decrease.

4. Before wilting was evident, there were marked reductions in apparent photosynthesis and transpiration, and an increase in respiration; in one case there was a 55 per cent. reduction in photosynthesis, a 65 per cent. reduction in transpiration, and a 62 per cent. increase in respiration. Stomata at this time appeared to be completely closed.

5. On several occasions fairly high rates of photosynthesis were obtained when the stomata appeared to be closed.

6. When the plants showed definite wilting, and the soil moisture was approximately at the wilting percentage, there was an 87 per cent. reduction in both photosynthesis and transpiration. It was not uncommon for wilted apple leaves to absorb from one to ten or more milligrams of carbon dioxide per 100 square centimeters of leaf surface per hour.

7. When water was applied to the soil in which wilted apple trees were growing, the leaves usually attained turgidity within three to five hours, depending upon their degree of wilting. They did not, however, recover to their original relationship with the controls in photosynthesis and respiration before two to seven days after the watering. Transpiration usually recovered about the same time as photosynthesis or slightly earlier.

8. The general trend of photosynthesis for the test plants was approximately the same in the field as in the environment-control chamber.

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FACTORS AFFECTING ROOT FORMATION OF *PHASEOLUS VULGARIS*

KENNETH V. THIMANN AND EUGENE F. POUTASSE

(WITH THREE FIGURES)

The successful rooting of leafy cuttings involves three physiologically distinct processes: initiation of root primordia; development of true roots from these initials; and maintenance of the cutting as an excised organ during the rooting.

The first of these, where it does not take place spontaneously, can be brought about in many plants by treatment with auxin. The second (outgrowth of the roots) involves the formation of cell-wall and protoplasm; it therefore requires the supply of carbohydrates and nitrogenous materials, together with such special substances as may be needed for root growth. Normally all of these materials would be supplied from the leaves or from storage in the stem. Too little attention has been paid up to now to the third process, the maintenance of the cutting, which, in view of the special conditions that obtain when leaves are severed from the plant, is of great importance. Thus, as pointed out by CHIBNALL (4), the detached leaf of many plants, including Phaseolus, undergoes a rapid and extensive proteolysis. The present experiments were, therefore, designed to shed some light on the second and third of these processes.

It was our intention to study the influence of nutrition, especially nitrogen nutrition, and other external chemical factors on the rooting and maintenance of leafy herbaceous material.

Since internal factors must also be considered in such a study, attention was paid to the rôle of auxin and also to the separate rôles of the leaves and the stem.

Materials and methods

Phaseolus vulgaris, the red kidney bean, was used throughout. The plants were raised in flats in the greenhouse and used at the age of about two weeks. The first simple leaf with its petiole (referred to as "leaf" throughout the text) was used in the majority of the experiments; isolated internodes, hypocotyls, and stem sections with attached leaves were also employed for comparison.

All solutions were made up with distilled water. Treatments were basal, and applied by immersion to a depth of about 2 cm. in the solution tested. The cuttings were supported by cotton plugs inserted into holes in thin wood covers, in such a manner that their bases dipped into beakers containing either water or the solution to be tested; the beakers were held in a wooden frame. In this way the bases were kept in darkness throughout

the experiment. The tops received the ordinary light conditions of the greenhouse. This was supplemented in the evening with a "sunlight" arc lamp to give a total constant day-length of fourteen hours since many experiments were carried out from November, 1939, to March, 1940. Each experiment lasted 15 to 20 days from the time of cutting. During the first twenty-four hours the cuttings were kept in auxin or water, the next twenty-four hours in the tested solution, and the following thirteen to eighteen days in distilled water.

Indole-3-acetic acid was employed as the auxin throughout; adenine was used as sulphate, choline and guanine as hydrochlorides. The weights of these substances given below, however, refer to the actual weight of base present.

Experimental results

The influence of auxin

The effects of treating the base of the petiole with indole-acetic acid of various concentrations are summarized in table I. The data are taken

TABLE I

Influence of treatment with indole-acetic acid for 24 hours on the survival and rooting of *Phaseolus vulgaris* cuttings*

Indole-acetic acid used per liter	Cuttings alive		Cuttings rooted		Average number of roots per rooted cutting	
	Water control	Auxin treated	Water control	Auxin treated	Water control	Auxin treated
mg.	%	%	%	%		
40	76	27	70	27		
10 (mean of 2 series)	75	46	68	46		
2 (mean of 2 series)	78	64	67	60		
0.5	25	29	4	29	10	40
0.1	61	46	54	40	63	44
0.025 (mean of 4 series)	80	69	41	37	23	57
0.025 (internode attached)	100	100	75	92	23	24

* All figures but the last refer to leaf cuttings; the last series have one complete internode attached. Results after 15 days in greenhouse.

from a number of separate experiments, each of which, however, included its own water controls.

It will be seen that the higher auxin concentrations caused a serious increase in mortality; even very low concentrations, in all but two of the experiments with the leaf-cuttings, somewhat reduced the number surviving. In only one of these two cases (0.5 mg. per liter) was there a significant increase in the percentage rooting.

On the other hand, practically all of the surviving cuttings, treated with auxin at 0.025 mg. per liter or higher, rooted; in the water controls, 10 to 80 per cent., or an average of about one-third of those surviving, did not root in the fifteen days. This may be seen by comparing the percentage alive with the percentage rooted in the same experiment. Further, in all but one of the seven experiments in which the number of roots was counted, auxin treatment greatly increased the number of roots per rooted cutting. This effect is not greatly dependent on auxin concentration. It is strongest in the two cases where rooting in water was poor. Examples of large increases in the number of roots formed may also be seen in tables III, VI and VII. To put it briefly, those cuttings which survive the auxin treatment show an increased root formation, but some cuttings are damaged even at concentrations as low as 1/40 mg. per liter. It is evident that this material has an unusually high sensitivity, to both the root-initiating and the toxic action of indole-acetic acid.

The notably lower mortality and higher rooting ability of the cuttings with attached stem is discussed in the next section.

Influence of the leaf and stem

In preliminary experiments it was found that the maintenance of single-leaf cuttings in water or nutrient solution depended on the amount of stem attached. With Phaseolus, which wilts easily, this phenomenon is very striking. The following figures show the percentage of cuttings which were evidently alive and not wilted when kept with their bases in distilled water for one week in the laboratory:

Cutting	Percentage survival
Leaf plus petiole (cut at A, see figure 1)	5
Leaf plus petiole plus node (cut at B)	22
Leaf plus petiole plus node plus half the next lower internode (cut at C)	30
Leaf plus petiole plus node plus whole next lower internode (cut at D)	67
Leaf plus petiole plus node plus whole next lower internode plus next lower node (cut at E)	78

Another comparison, with somewhat younger plants, done in the greenhouse, is presented in table II. Though the survival is higher and thus more normal, the beneficial influence of the added stem section is still clear. Since the internode by itself does not survive appreciably (column 4) it is the combination of leaf with stem tissue which is most viable. The results in nutrient solution are similar to those in water, so that the effect cannot be ascribed to simple nutritional deficiency.

Root formation shows the same response as survival. The effect of an attached internode is shown in table III. The cuttings were first treated

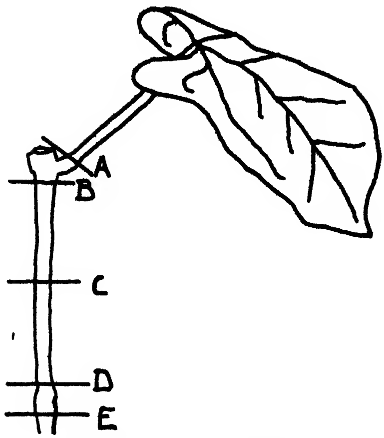

FIG. 1. Sketch showing points at which cuttings were taken.

TABLE II

SURVIVAL OF VARIOUS TYPES OF PHASEOLUS CUTTINGS AFTER 15 DAYS IN THE GREENHOUSE, IN WATER OR IN NUTRIENT*

	PERCENTAGE OF CUTTINGS ALIVE				
	LEAF ONLY; (CUT AT A) MEAN OF 3 EXPTS.	LEAF PLUS NODE; (CUT AT B) 1 EXPT.	LEAF PLUS NODE PLUS INTERNODE; (CUT AT D) 1 EXPT.	ISOLATED (LEAFLESS) INTERNODE; MEAN OF 2 EXPTS.	ISOLATED HYPO-COTYL; MEAN OF 2 EXPTS.
	%	%	%	%	%
In water	65	70	85	2	17
In nutrient solution	67	90	90	2	12

* Hoagland's solution, quarter strength. For points at which cutting was made, see figure 1.

TABLE III

ROOTING AND MORTALITY AFTER 16 DAYS IN WATER IN THE GREENHOUSE. AUXIN TREATMENT 0.025 MG. PER LITER

	PERCENTAGE ROOTED		NUMBER OF ROOTS PER ROOTED CUTTING		PERCENTAGE ALIVE	
FIRST 24 HOURS IN:	WATER	AUXIN	WATER	AUXIN	WATER	AUXIN
	%	%			%	%
Leaf (cut at A)	23	8	3	47	92	80
Leaf plus internode (cut at D)	69	92	21	22	100	100

basally with water or auxin (0.025 mg. per liter) for 24 hours, then kept with their bases in water for 16 days and roots counted. When the in-

ternode is absent, auxin increases the number of roots per rooted cutting. It also evidently causes some toxicity, as shown by reduced percentage of rooting and increased mortality. When the internode is attached this toxicity disappears and at the same time the percentage of cuttings rooted, either with or without auxin, is greatly increased and survival becomes complete.

The explanation for this influence of attached stem sections in maintaining the cutting alive cannot as yet be given, but it seems highly probable that it is due to a contribution of some organic substance, or substances, by the stem.

After-treatment of the cuttings

Mineral nutrients.—In maintaining cuttings in solution it was first necessary to know whether or not they required aeration. In comparative experiments with leaves, internodes, and hypocotyls the influence of aerating the water or nutrient solution was found to be negligible. In most instances, indeed, the stream of air so promoted the growth of microörganisms that the cuttings became infected. Aeration was therefore not adopted.

The effect of providing nutrients was tested in a number of experiments. Hoagland's solution (7) at one quarter strength was found satisfactory. It maintained the roots in white and active condition, as compared to the evident browning after 15 days in water. On the other hand the solution, if anything, inhibited root initiation. In one experiment leaves, with and without auxin treatment, rooted 42 and 45 per cent., respectively, in nutrient solution; those kept in water rooted 55 and 50 per cent., respectively.

TABLE IV

Influence of nutrient salts. Auxin 0.025 mg. per liter

First 24 hours	Nutrient solution second 24 hours	Cuttings rooted	Mean number of roots per rooted cutting	Total number of roots on 30 cuttings
		%		
Water Auxin	Water	60 60	27 29	520
Water Auxin	$Ca(NO_3)_2$ 200 mg./l.	47 47	26 35	407
Water Auxin	KH_2PO_4 20 mg./l.	60 27	45 23	487
Water Auxin	KH_2PO_4 200 mg./l.	27 53	13 39	369
Water Auxin	KNO_3 50 mg./l.	47 73	82 23	827
Water Auxin	KNO_3 500 mg./l.	67 97	21 41	785

This slight retarding effect of the complete nutrient solution is, however, in sharp contrast with the behavior of the individual salts separately. Rooting was markedly inhibited by $MgSO_4$ and by $Ca(NO_3)_2$ at the same concentration as in the nutrient solution; but strongly promoted by KNO_3 and in a few experiments weakly by KH_2PO_4. The most powerful inhibition was exerted by $MgSO_4$, which at M/3000 (82 mg. per liter) completely prevented any rooting of leaves and increased the mortality from 13 to 33 per cent. A comparison of the other salts is given in table IV. The beneficial influence of KNO_3 at relatively high concentrations can best be seen from the total number of roots in the last column.

The data in table IV, showing the increased root formation when cuttings were supplied with KNO_3, were extended by comparison with another source of available nitrogen, namely ammonium sulphate. Table V shows that

TABLE V

COMPARISON OF NITRATE WITH AMMONIUM. INDOLE-ACETIC ACID 0.5 MG. PER LITER. RESULTS AFTER 15 DAYS IN GREENHOUSE

1ST 24 HOURS IN	SECOND 24 HOURS		ROOTED	TOTAL NUMBER OF ROOTS ON 24 CUTTINGS
			%	
Water	Water		4	100
Auxin			28	202
Water	KNO_3	20 mg./l.	8	160
Auxin			25	270
Water	KNO_3	200 mg./l.	25	280
Auxin			33	270
Water	$(NH_4)_2SO_4$	2 mg./l.	8	35
Auxin			8	35
Water	$(NH_4)_2SO_4$	20 mg./l.	0	0
Auxin			0	0

even at 2 mg. per liter (M/66000) rooting is strongly inhibited, while 20 mg. per liter and all higher concentrations completely prevent all rooting. KNO_3, on the other hand, is again effective.

ORGANIC NITROGEN.—Organic nitrogenous compounds, especially those known to be present in leaves, are of particular interest in this connection. The analyses of VICKERY (8) on alfalfa show that, apart from amino-acids, the most important nitrogenous constituents of the leaves are asparagine, adenine, stachydrin, and choline. Since the bean is also a legume, these substances were considered the most important to study. Stachydrin was, however, omitted, and instead some other purines and nicotinic acid were used.

The results showed considerable variation from experiment to experi-

ment, and will therefore be given in abbreviated form. The controls themselves varied markedly, perhaps due to small differences in humidity and lighting which are unavoidable under greenhouse conditions. There is also some evidence that when cuttings are taken late in the day their subsequent survival is impaired. In each of the experiments, however, the cuttings for all treatments were taken at one time. Two additional reasons add to the difficulty of drawing conclusions. One is that some substances showed effects mainly when the cuttings had been pre-treated with auxin; *i.e.*, they enhanced the effectiveness of the auxin treatment, while others were more effective when auxin had not been used. The other is that in some instances the percentage of cuttings which rooted was increased, while the total number of roots formed on all the cuttings was not. The opposite also occurred. For example, the best single effect on root number was obtained with guanine 20 mg. per liter, which gave 583 roots per 15 leaf cuttings against 113 in the controls, *i.e.*, an increase of 31 roots per cutting. The highest increase in percentage rooting of leaf cuttings was, however, in one of the experiments with nicotinic acid 20 mg. per liter, which gave 67 per cent. rooting as against only 20 per cent. in the controls. In spite of these uncertainties, the great effectiveness of adenine and asparagine in promoting rooting may be regarded as fully established, while that of the other substances tested is at least highly probable.

Adenine gave the most consistent results. Out of 6 experiments, each one including both water and auxin pre-treatment (12 comparisons), adenine increased the percentage rooting and the total number of roots in every case. The data are summarized in figure 2, which shows the increases above the controls for each of three concentrations used. Concentrations below 50 mg. per liter showed no consistent effectiveness. Apparently the actual concentration which causes the best effect depends on whether auxin was used or not, the higher concentrations being more effective with auxin pre-treatment, the lower without. It is tempting to ascribe this to a "limiting factor" effect such as is discussed by WENT and THIMANN (9); the data obtained with other substances, however, do not bear this out.

The effects of adenine were of good magnitude. In one experiment 4 per cent. rooted in water controls and 42 per cent. in those treated with adenine 50 mg. per liter; in another 38 per cent. rooted in auxin controls and 77 per cent. when auxin was followed by adenine 200 mg. per liter. Also, in spite of the variation between control values in different experiments, the results are statistically fully significant. The mean percentage rooting of all *controls* in the adenine experiments (both water and auxin treated) was 22.5 ± 0.8, while the mean percentage rooting of all *adenine-treated* cuttings (both water and auxin pretreated) was 41.8 ± 0.5. As to root number, the controls averaged, per 15 cuttings, 142 ± 8 roots, those

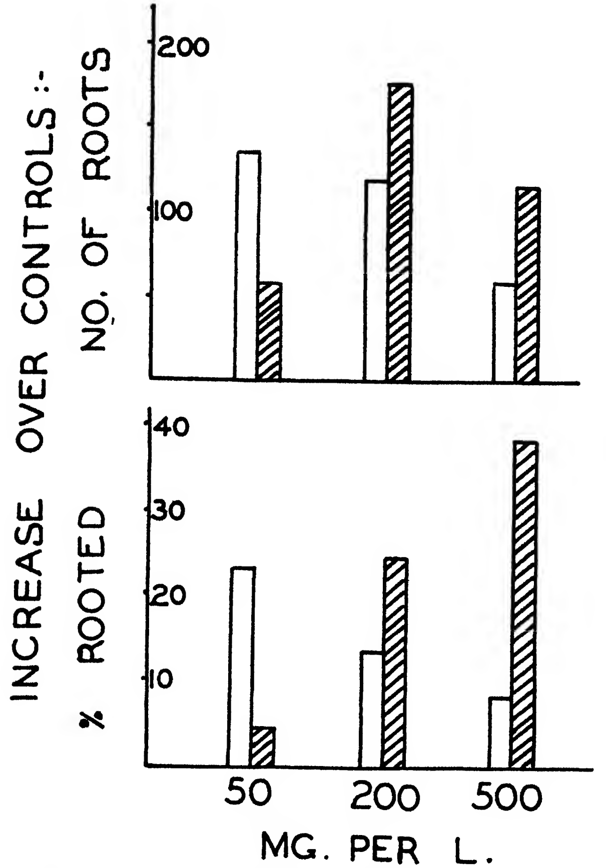

FIG. 2. The effect of adenine as after treatment.

Ordinate: increase in root number and in rooting percentage as a result of adenine treatment.

Abscissa: concentration of adenine.

Shaded columns: cuttings pretreated with auxin.

White columns: no auxin.

Data for 50 mg. per liter the mean of two experiments; those for 200 mg. per liter the mean of three.

treated with adenine 252 ± 7. Examples of single experiments are also included in tables VII, VIII, and IX.

The higher rooting of cuttings with attached internode (such as were used for table III) apparently precludes any further increase by adenine. One such experiment was carried out, but both the controls and those given adenine rooted 85 per cent. and both averaged 18 roots per cutting.

The results of two similar experiments with asparagine are given in table VI. High concentrations were not practicable on account of infection.

TABLE VI

INFLUENCE OF ASPARAGINE (5 MG. PER LITER)*

First 24 hours	Second 24 hours			
	Water		Asparagine	
	Percentage rooting	Total number of roots on 15 cuttings	Percentage rooting	Total number of roots on 15 cuttings
	%		%	
Water	8	8	13	45
Auxin	16	150	21	112
Water	54	509	69	726
Auxin	38	254	62	516

* Auxin: indole-acetic acid 0.1 mg. per liter. Results after 15 days in greenhouse. Two separate experiments, each with water and auxin treatment.

The optimum concentration has evidently been covered, however, since 50 mg. per liter was less effective than 5 mg. per liter, which was used for both series of table VI. Seven of the eight comparisons show real increases. The mean increase in percentage rooting caused by asparagine is 12 ± 1.3; the mean increase in root number is 120 ± 20, so that the data are fully significant. On the whole, asparagine 5 mg. per liter was nearly as effective as adenine.

Two experiments with guanine are summarized in figure 3. The percentage of cuttings rooting was increased markedly in five out of the six comparisons, but the total number of roots was increased only when auxin had not been supplied. The increases were, however, very large.

Results with uracil, 0.1 and 5 mg. per liter, were somewhat erratic, but

TABLE VII

INFLUENCE OF YEAST NUCLEIC ACID AND OF ADENINE*

First 24 hours	Second 24 hours				Type of cutting
	Water	Nucleic acid 200 mg./l.	Nucleic acid 500 mg./l.	Adenine 500 mg./l.	
Water	9	0	13	67	Leaf
Auxin	55	20	98	171	
Water	198	175			Leaf
Auxin	261	520			
Water	255	315	375		Leaf and one internode
Auxin	300	285	420		

* All data the total number of roots on 15 cuttings. Auxin: indole-acetic acid 0.025 mg. per liter. Results after 15 days in greenhouse.

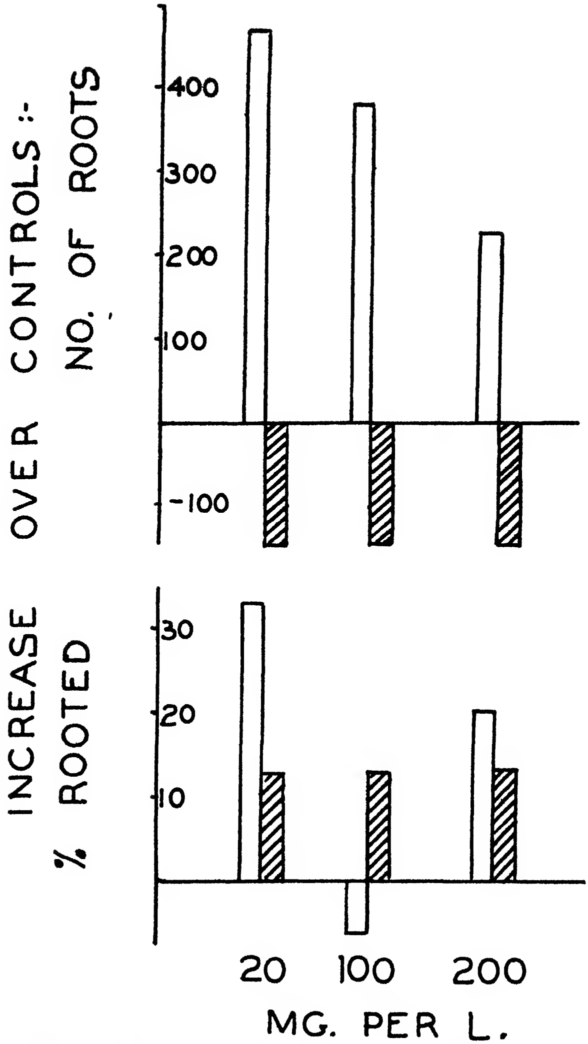

FIG. 3. The effect of guanine as after-treatment. Legends as for figure 2. Each concentration one experiment only. The number of roots is increased only when auxin is not used.

there was a marked increase in root number in the cuttings pretreated with auxin (504 on 15 controls, 771 on 15 uracil-treated).

It was thought that the beneficial effect of the purines might be combined with that of phosphate by administering nucleic acid. Yeast nucleic acid was therefore tested at 20 to 500 mg. per liter. The higher concentrations appeared to show a real increase in root number (table VII) but no increase in percentage rooting. Adenine in the same experiment was much more

effective. Cuttings with internode attached also showed a response to nucleic acid at 500 mg. per liter. Infection of the bases of the cuttings was, however, very troublesome at these high concentrations.

An experiment with choline is presented in table VIII. In the absence

TABLE VIII

INFLUENCE OF CHOLINE AND OF ADENINE. AUXIN 0.5 MG. PER LITER. RESULTS AFTER 15 DAYS IN GREENHOUSE

FIRST 24 HOURS	PERCENTAGE OF ROOTING			
	WATER	CHOLINE 5 MG./L.	CHOLINE 50 MG./L.	ADENINE 50 MG./L.
	%	%	%	%
Water	4	25	25	42
Auxin*	29	8	0	25
	TOTAL ROOTS ON 15 CUTTINGS			
Water	6	89	140	250
Auxin*	144	69	0	119

* The auxin data are included for the sake of completeness, although the concentration used was evidently too high.

TABLE IX

INFLUENCE OF ADENINE. AUXIN 0.1 MG. PER LITER. RESULTS AFTER 15 DAYS IN GREENHOUSE

FIRST 24 HOURS	PERCENTAGE OF ROOTING		
	WATER	ADENINE 50 MG./L.	ADENINE 200 MG./L.
Water	54	62	70
Auxin	40	46	77
	TOTAL ROOTS ON 15 CUTTINGS		
Water	509	535	831
Auxin	255	494	646

of auxin treatment, both total root number and percentage rooting are clearly increased, but the combination of choline with auxin pre-treatment seems to be very damaging. This may be due to the rather high auxin concentration used in this particular experiment. For comparison, the data for adenine (50 mg. per liter) obtained in the same experiment are included. They show the same tendency to fall where auxin was used, but they also show how large an effect can be exerted by adenine.

Nicotinic acid, at 4 to 100 mg. per liter, produced small increases in the percentage rooting and the number of roots formed, mainly when auxin was not supplied, but the response was variable and no certain conclusions can be drawn. It may also be noted that malate M/1000, was completely without effect. This is of interest since malic acid appears to function in the oat coleoptile as part of the auxin complex.

Discussion

The powerful influence of a section of stem, both in maintaining the cuttings and promoting rooting, shows that the stem exerts an important influence on the leaf. Because of the extensive proteolysis initiated in leaves on their removal from the stem, even in light (**4**, table 91), the inference may be made that the presence of the stem in some way prevents this proteolysis. Probably, therefore, maintenance of the isolated leaf is a function of the degree to which proteolysis is prevented. In view of the clear effects exerted by nitrate and organic nitrogen in the present experiments, it is suggested that the stem contributes to the leaves an available form of nitrogen. Indeed, BOTH (**3**) has shown that nitrogenous material can be readily transported from a stem section into an attached leaf, provided only that the water-vapor tension is below saturation.

It is of interest that besides nitrate and asparagine, the purines, adenine especially, are so effective. The importance of adenine for the synthesis of nuclear material and of coenzymes, and the ease with which asparagine is metabolized in the plant, doubtless account for the fact that these two are the most effective substances of those tested. The activity of adenine on the expansion of leaf-blades has been reported by BONNER and HAAGEN-SMIT (**2**), and apparently in some plants the effect is exerted on vegetative growth generally (**1**). The range of active concentrations, however, in their continuous watering experiments is much lower than those effective in the present short-time treatments. More closely related, perhaps, are the interesting recent observations of DOAK (**5**), who found a variety of nitrogenous compounds, mostly amino-acids and purines, to promote rooting in cuttings of a Rhododendron. However, while asparagine was one of the most active substances he tested (in agreement with our data), adenine and guanine were much less so. The general point that small quantities of organic nitrogen enabled considerable rooting to occur remains clear in both cases.

In view of the effectiveness of nitrate, it is possible that the improvement in rooting of spruce cuttings caused by nutrient solution (**6**) is due to the nitrate in the solution, particularly since (as shown above) Ca and PO_4 have little influence, while $MgSO_4$ has even the opposite effect.

The usual methods of propagation by cuttings entail more or less com-

plete nitrogen starvation of the material. The present results suggest that, for some plants at least, this procedure might well be radically revised.

Summary

The rooting of isolated leaves of *Phaseolus vulgaris*, with or without attached stem sections, has been studied.

1. The survival of the cuttings after removal from the plant is greatly improved by presence of a portion of stem, and varies roughly with the amount of stem attached.
2. The material is extremely sensitive to auxin, a concentration of 0.025 mg. indole-acetic acid per liter being the highest that can be applied (for 24 hours) without producing injury. However, auxin treatment in nearly all experiments considerably increased the number of roots formed per rooted cutting.
3. Rooting is slightly depressed by a complete nutrient solution, or by the Ca or Mg salts composing it, but is strongly promoted by potassium nitrate. Ammonium sulphate, even at high dilution, is inhibitory. The growth and maintenance of roots already formed is, however, improved by the complete nutrient solution.
4. Of the organic nitrogen sources studied, adenine was the most effective, greatly increasing both the percentage of cuttings which rooted and the number of roots formed by each rooted cutting. Asparagine, guanine, choline, and uracil were also effective to a lesser extent, in approximately that order, while yeast nucleic acid and nicotinic acid showed slight but variable effects.

It is concluded that the nitrogen nutrition of the isolated leaf is of great importance in determining both its survival and its root formation.

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TRANSFORMATION OF SUGARS IN EXCISED BARLEY SHOOTS

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(WITH TWO FIGURES)

Introduction

Nelson and Auchincloss (**10**) working with potato tissue showed that sucrose is synthesized from glucose or fructose rather than from starch as has been supposed by some investigators. That such a conversion occurs in plant metabolism has also been shown by Virtanen and Nordlund (**14**) in red clover and wheat plants, and more recently by Hartt (**3**) in detached sugar cane leaves.

Since sucrose, consisting of a glucose and fructose molecule, can readily be synthesized from either of these monosaccharides, it may be assumed that such synthesis involves a preliminary conversion of each of these hexose sugars into the other. The fructose component of the sucrose molecule exists in the furanose form (unstable γ form with the five-membered ring) and is different from the six-membered fructose (pyranose configuration) from which it can be synthesized. The plant apparently also possesses a mechanism to render this transformation possible. The fact that sucrose is synthesized when plants are artificially supplied with glucose or fructose can serve indirectly in the study of transformation of other hexoses in the plant into these monosaccharides. If, for example, synthesis of sucrose is observed after supplying the plant with mannose, it could be assumed that a preliminary conversion of the latter into glucose and fructose must take place before the synthesis of sucrose occurs. The hexoses that are known to occur naturally in plants are: d-glucose, d-fructose, d-mannose, and d-galactose; of these the last two have not been detected in the free state and are found in plants only as units in polysaccharides. If, as it is generally assumed, some form of glucose is the first product of photosynthesis, the plant should possess a mechanism whereby this molecule is transformed into mannose or galactose before the synthesis of the corresponding polysaccharide can be possible.

In this investigation, using the infiltration method (**8**), the following compounds were artificially supplied to barley plants: d-glucose, d-fructose, d-mannose, d-galactose, sucrose, lactose, maltose, l-arabinose, d-xylose, mannitol, sorbitol, gluconic acid, pyruvic acid, and glyceric aldehyde. The increase of sucrose was observed after certain periods of time to determine the ability of the plant to transform these compounds into glucose or fructose and, if possible, to ascertain the mechanism of this transformation.

Experimental methods and results

It is well known that detached leaves or plants detached from their roots are capable of carrying on metabolic activities for a considerable length of time. KURSANOV (8) utilized this fact to study the synthetic and hydrolytic actions of invertase in living plant tissues by his "vacuum infiltration" method. This method was effectively used in our investigation of sugar transformations in barley plants. Barley plants were grown in half strength HOAGLAND's culture solution for about 3 weeks. The plants were cut above the roots (only the shoots were used) and 5-gm. samples of the fresh material were immersed into beakers containing 5 per cent. sugar solution. The beakers with contents were placed into a desiccator and evacuated to about 20 mm. of mercury for 5 minutes. After evolution of the air bubbles from the leaves had ceased, air was slowly re-admitted into the desiccator. The intercellular spaces were thereby replaced by the sugar solution. The plant material was then taken out of the beakers, and allowed to remain in the dark at about 22° C. in an atmosphere saturated with water vapor for a certain period of time. The material was then thoroughly washed with water, the soluble sugars extracted with boiling 80 per cent. alcohol and analyzed for reducing sugars by oxidation with ferricyanide and titration with ceric sulphate (6, 7) before and after hydrolysis with invertase. Sucrose was determined by the difference. Blank samples of 5 gm. fresh weight of the same plant material infiltrated with water were run simultaneously. In all cases the samples were run in duplicate. The results are expressed on the basis of fresh weight.

Preliminary experiments showed that barley samples infiltrated with 5 per cent. solutions of glucose, fructose, mannose, galactose, lactose, and maltose, and allowed to remain for several hours showed a considerable gain in sucrose over the blank samples infiltrated with water. This indicated synthesis of sucrose by the plant from these sugars. Since the synthesis was measured by difference in sucrose between the sugar and the water infiltrated samples, the production of sucrose in these experiments was subject to doubt. It should be borne in mind that besides synthesis of sucrose, other metabolic processes go on simultaneously in the plant. While synthesis of sucrose is taking place, glucose or fructose is being used up in respiration, and in order to maintain a proper state of equilibrium a certain amount of sucrose is hydrolyzed back into these monosaccharides. In the samples infiltrated with solutions of monosaccharides, there is an excess of hexose sugars available for respiration. The hydrolysis of sucrose into glucose and fructose will therefore tend to decrease, and the equilibrium represented by the equation: glucose + fructose $\rightleftarrows$ sucrose will be shifted to the right. In the water infiltrated samples the tendency of the sucrose to hydrolyze is greater, and the equilibrium will be shifted to the left. The

difference in sucrose content between the sugar infiltrated sample and the blank would therefore not represent the true value of the amount of sucrose synthesized. To avoid this difficulty a procedure was adopted to deplete the sucrose to a low level, by respiring the barley plants for a certain length of time previous to infiltration with the sugar solutions.

The tendency of sucrose to be depleted first during respiration, while the reducing sugars are maintained more or less at a constant level, is shown by the results in table I.

TABLE I

COMPARISON OF SUCROSE AND REDUCING SUGARS IN EXCISED BARLEY SHOOTS AFTER RESPIRATION FOR 24 AND 48 HOURS IN THE DARK AT 22° C.

SAMPLE	DESCRIPTION OF SAMPLE	REDUCING SUGARS	TOTAL SUGARS	SUCROSE	SUCROSE PER 5 GM. OF FRESH MATERIAL
		%	%	%	mg.
1	Initial untreated	0.87	1.10	0.22	11.0
2	Depleted (24 hours) in water	0.90	0.90	0.0	0.0
3	Depleted, then water infiltrated 24 hours	0.77	0.77	0.0	0.0
4	Depleted, then infiltrated 5% glucose 24 hours	1.09	1.43	0.32	16.0

The untreated sample, no. 1, analyzed immediately after harvesting, contained 0.87 per cent. reducing sugars and 0.22 per cent. sucrose or 11.0 mg. per 5 gm. of fresh weight. After the plants respired in the dark for 24 hours the reducing sugars remained at about the same order of magnitude, 0.9 per cent. but the sucrose was all depleted (no. 2 sample). When the respired sample, no. 3, was infiltrated with water and allowed to remain for another 24 hours, the reducing sugars came down to 0.77 per cent. A decrease in the reducing sugars could be observed only after all the sucrose was exhausted. Sample no. 4, which was infiltrated with 5 per cent. glucose after all the sucrose was depleted, synthesized 16.0 mg. of sucrose per 5 gm. of fresh weight in 24 hours and contained 1.09 per cent. reducing sugars.

Table II represents results of experiments with barley shoots which respired in the dark for 24 hours to a low level of sucrose of 1.0 mg. per 5 gm. of fresh weight. Samples of these plants were then infiltrated with 5 per cent. solutions of glucose, fructose, mannose, galactose, and arabinose and allowed to remain for 18 hours. As shown in the table, sucrose was formed from glucose, fructose, mannose, and galactose but not from arabinose. The relative rate of sucrose synthesis in the plant from these sugars with

TABLE II

SUCROSE SYNTHESIS IN SUCROSE DEPLETED BARLEY SHOOTS AFTER INFILTRATION WITH 5 PER CENT. SOLUTIONS OF VARIOUS MONOSACCHARIDES AND ALLOWED TO REMAIN FOR 18 HOURS AT 22° C.

SAMPLE	DESCRIPTION OF SAMPLE	REDUCING SUGARS	TOTAL SUGARS	SUCROSE	SUCROSE PER 5 GM. OF FRESH MATERIAL	RELATIVE RATE OF SUCROSE FORMATION
		%	%	%	mg.	
1	Initial untreated	0.86	1.14	0.27	13.5	
2	Depleted (24 hours) in water	0.78	0.80	0.02	1.0	
3	Depleted, then water infiltrated 24 hours	0.58	0.60	0.02	1.0	
4	Glucose infiltrated	0.50	0.97	0.45	22.5	100.0
5	Fructose infiltrated	0.50	0.83	0.31	15.5	67.5
6	Mannose infiltrated	1.64	1.89	0.24	12.0	51.2
7	Galactose infiltrated	1.73	1.88	0.14	7.0	27.9
8	Arabinose infiltrated	1.52	1.52	0.0	0.0	

respect to glucose was as follows: fructose, 67.5 per cent.; mannose, 51.3 per cent.; galactose, 27.9 per cent.

Figure 1 gives the results from 8 samples of barley plants which were infiltrated with 10 per cent. glucose solution, analyzed for sucrose immediately, and then the synthesis of sucrose followed at different intervals for 48 hours.

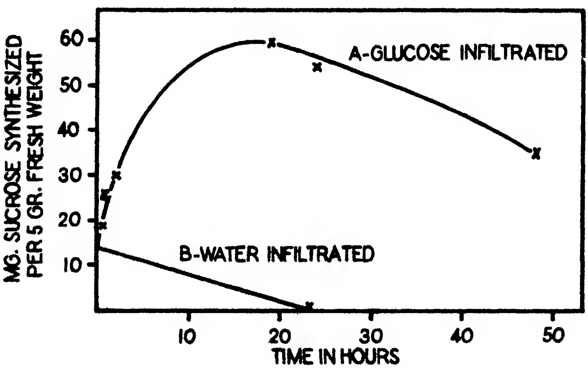

FIG. 1. Synthesis of sucrose from glucose.

As seen from curve A the maximum amount of sucrose was synthesized after about 18.5 hours (59 mg. per 5 gm.). From then on the amount of synthesis gradually diminished. Line B shows the depletion of sucrose. The entire content of sucrose was depleted from an equivalent sample of barley within 24 hours. Curve A therefore represents the synthesis of sucrose above the amount depleted during respiration.

The synthesis of sucrose from galactose is shown in figure 2.

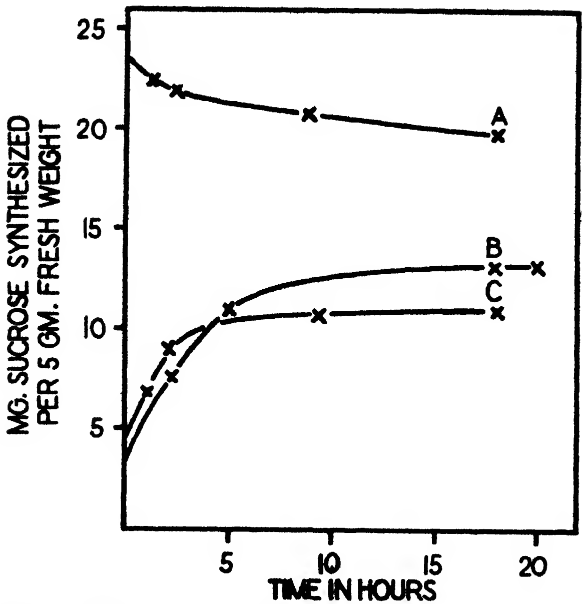

FIG. 2. Synthesis of sucrose from galactose during 20 hours. A, not depleted of sucrose before infiltration. B, depleted. C, recrystallized galactose and sample depleted before infiltration.

Galactose was infiltrated into one set of barley samples, originally containing 23.5 mg. per 5 gm. of fresh weight, analyzed immediately and the synthesis of sucrose followed for 18 hours. Since the rate of sucrose formation from galactose is not very great (shown in table II, 27.9 per cent. as from glucose) it did not exceed the hydrolysis which was apparently simultaneously taking place during respiration. Therefore, not a gain but rather a slight loss in sucrose could be observed, as shown by slightly downward curve A. In a second set of barley plants, however, in which the sucrose had been previously exhausted to a low level, a distinct gain of sucrose could be observed from galactose, as shown by curve B.

Since commercial galactose is usually made by hydrolysis of lactose, which consists of one molecule of galactose and one molecule of glucose, it was tested for purity with respect to glucose contamination A fermentation test with a pure culture of yeast (*Torula monosa*) showed that the galactose contained almost 1 per cent. glucose. The commercial galactose was therefore purified by dissolving in water and fermenting the glucose and then crystallizing from alcohol. A fermentation test on this purified galactose showed no glucose contamination. Curve C shows sucrose formation when purified galactose was used. This eliminates the possibility that the sucrose could have been formed from the small amount of glucose found as an impurity and not from the galactose.

Table III shows the results of experiments in which barley plants were infiltrated with solutions of xylose, maltose, lactose, mannitol, sorbitol, glyceric aldehyde, and the sodium salts of gluconic and pyruvic acids. In these experiments the plants were depleted of sucrose in the usual manner before infiltration with the solutions. A sample of plants was also infiltrated with glucose in order to determine the rate of sucrose formation from the particular sugar with respect to glucose.

TABLE III

SUCROSE SYNTHESIS IN SUCROSE DEPLETED BARLEY SHOOTS AFTER INFILTRATION WITH 5 PER CENT. SOLUTIONS OF VARIOUS SUGARS OR RELATED SUGAR COMPOUNDS AND ALLOWED TO REMAIN FOR 20 HOURS AT 22° C.

SAMPLE	DESCRIPTION OF SAMPLE	REDUCING SUGARS	TOTAL SUGARS	SUCROSE	SUCROSE PER 5 GM. OF FRESH WEIGHT	RELATIVE RATE OF SUCROSE FORMATION
		%	%	%	mg.	
1	Initial—after depletion	1.23	1.43	0.19	9.5	
2	Depleted, then water infiltrated (20 hours)	0.79	0.90	0.10	5.0	
3	Glucose infiltrated	1.80	2.58	0.74	37.0	100.0
4	Xylose infiltrated	2.34	2.47	0.12	6.0	
5	Maltose infiltrated	2.09	2.90	0.77	38.5	105.0
6	Lactose infiltrated	2.03	2.60	0.54	27.0	68.8
7	Mannitol infiltrated	0.79	0.92	0.12	6.0	
8	Sorbitol infiltrated	0.60	0.67	0.07	3.5	
9	Glyceric aldehyde	1.00	1.40	0.38	19.0	43.8
10	Gluconic acid (Na salt)	1.20	1.37	0.16	8.0	
11	Pyruvic acid (Na salt)	0.70	0.88	0.17	8.5	

Sucrose was formed in the barley plants from maltose, lactose, and glyceric aldehyde. The rate of sucrose synthesis from these sugars with respect to glucose is 105 per cent., 68.8 per cent. and 43.8 per cent. respectively. It is of interest to note that the disaccharides, maltose and lactose, are used by the plants for the synthesis of sucrose and that maltose is utilized to about the same extent as glucose. Apparently the barley plant possesses enzymes which readily hydrolyze these disaccharides to their respective monosaccharides, which are then used for sucrose synthesis. Xylose, mannitol, sorbitol, and the salts of gluconic and pyruvic acids were not utilized. The utilization of a pentose sugar for sucrose synthesis would require an additional carbon atom for the preliminary formation of a hexose. Evidently the barley plant does not possess a mechanism for such a transformation. There are apparently also no enzymes which could reduce sugar acids or oxidize sugar alcohols to their corresponding aldohexoses. The formation of a considerable amount of sucrose from glyceric aldehyde shows that the plant is able to condense this tricarbon compound to glucose or fructose.

In table IV the results with etiolated plants are presented. The plants were germinated and grown in a culture solution in the dark for about 2 weeks; they were light yellow and absolutely devoid of chlorophyll.

TABLE IV

SUCROSE SYNTHESIS IN ETIOLATED BARLEY SHOOTS INFILTRATED WITH 5 PER CENT. SOLUTIONS OF VARIOUS SUGARS, AFTER RESPIRATION IN THE DARK FOR 24 HOURS, AND THEN ALLOWED TO REMAIN FOR 18 HOURS AT 22° C.

SAMPLE	DESCRIPTION OF SAMPLE	REDUCING SUGARS	TOTAL SUGARS	SUCROSE	SUCROSE PER 5 GM. OF FRESH WEIGHT
		%	%	%	*mg.*
1	Initial untreated	0.50	0.50	0.0	0.0
2	Depleted for 24 hours	0.42	0.42	0.0	0.0
3	Glucose infiltrated	1.30	1.83	0.50	25.0
4	Sucrose infiltrated	1.14	1.66	0.49	24.5
5	Maltose infiltrated	0.87	1.41	0.51	25.5
6	Galactose infiltrated	1.30	1.58	0.27	13.5

Sucrose formation takes place in barley plants which are devoid of chlorophyll. The etiolated plants did not contain any sucrose (sample no. 1). Sample no. 4, infiltrated with sucrose, did not raise the sucrose content above the one infiltrated with glucose; instead, it raised the value of reducing sugars. As shown in the table IV, the value of the reducing sugars in the blank sample after respiration for 24 hours was 0.42 per cent. After being infiltrated with glucose and allowed to remain for 18 hours, the respired barley plants contained 0.50 per cent sucrose and 1.30 per cent. glucose. The plants infiltrated with sucrose and allowed to remain for the same period of time, contained about the same amount of sucrose, 0.49 per cent., and 1.14 per cent. reducing sugars. It appears that an excess of reducing sugars induces synthesis of sucrose, while an excess of sucrose favors the reverse process, hydrolysis.

In order to show whether the synthesis of sucrose from a monosaccharide requires oxygen, the following experiment was carried out: barley samples infiltrated with 5 per cent. glucose were placed in 3 desiccators. The first contained air saturated with water vapor, in the second the air was replaced by oxygen, and in the third by nitrogen. The plants were allowed to remain in the dark for 24 hours. Originally they contained 47.5 mg. of sucrose per 5 gm. of fresh weight, and respired for 36 hours to a level of 19.5 mg. The results are shown in table V.

Sample no. 4, in the air, synthesized 58.5 mg. per 5 gm. of tissue. When the air was replaced by nitrogen only 22.5 mg. of sucrose per 5 gm. of tissue were synthesized, a value slightly higher than the blank, 19.5 mg. Sample no. 3, in pure oxygen synthesized 63.0 mg. of sucrose per 5 gm. of tissue, a

TABLE V

SUCROSE SYNTHESIS IN BARLEY SHOOTS INFILTRATED WITH 5 PER CENT. SOLUTIONS OF GLUCOSE AND EXPOSED TO AN ATMOSPHERE OF AIR, NITROGEN, AND OXYGEN FOR 24 HOURS AT 22° C.

SAMPLE	DESCRIPTION OF SAMPLES	REDUCING SUGARS	TOTAL SUGARS	SUCROSE	SUCROSE PER 5 GM. OF FRESH WEIGHT
		%	%	%	mg.
1	Initial after depletion (in air)	1.37	1.78	0.39	19.5
2	Nitrogen	2.00	2.47	0.45	22.5
3	Oxygen	2.03	3.36	1.26	63.0
4	Air	2.10	3.33	1.17	58.5

value slightly higher than in air. This indicates that the synthesis of sucrose is an aerobic, or at least partly aerobic, process.

Invertase is generally considered to be specific for sucrose. The question of strict specificity of this enzyme is of prime importance in this investigation, since the conclusion that sucrose is synthesized from supplied monosaccharides rests entirely upon this assumption. There is a possibility that a compound other than sucrose might be formed which could be hydrolyzed by invertase. For example, when the plant is supplied with mannose or galactose, some non-reducing disaccharide might be formed which would also be subject to the attack of invertase. The release of free monosaccharides would produce a high reducing value similar to the hydrolysis of sucrose. If the reducing value, after invertase hydrolysis, is in reality due to sucrose (since sucrose contains a molecule of fructose) half of the increase of that value should be approximately equal to a corresponding increase in fructose. To verify this point, barley plants were infiltrated with 5 per cent. solutions of glucose, mannose, and galactose, and the increase in sucrose obtained by the invertase method was compared with the increase in fructose determined by ROE's method (**11**).

The results in table VI show that, after supplying galactose or mannose, the increase in the reducing value, by hydrolysis with invertase, corresponds approximately with the increase in fructose. This indicates that the carbohydrate synthesized is sucrose.

Discussion

The synthesis of sucrose in barley when either glucose, fructose, mannose, or galactose is supplied, indicates that there is a mechanism in the plant, which renders the conversion of these monosaccharides possible. HARTT (**4**) working with blades of sugar cane found that mannose was not utilized to form sucrose. The power of interconversion of the different monosaccharides apparently is dependent on the enzymic system and varies

TABLE VI

SUCROSE SNYTHESIS IN SUGAR DEPLETED BARLEY SHOOTS, ESTIMATED FROM INCREASE IN TOTAL REDUCING VALUE AND FRUCTOSE CONTENT AFTER HYDROLYSIS.

Sample	Description of sample	Time interval after infiltration	Reducing sugars	Total sugar	Sucrose	Sucrose per 5 gm. fresh weight	Fructose per 5 gm. fresh weight	Net increase of fructose per 5 gm. fresh weight
		hrs.	%	%	%	*mg.*	*mg.*	*mg.*
1	Initial—untreated		0.43	0.77	0.32	16.0	10.8	
2	Depleted—36 hours		0.37	0.37	0.0	0.0	6.0	
3	Galactose infiltrated	1	1.03	1.10	0.07	3.5	6.5	0.5
4	Galactose infiltrated	23	0.83	1.07	0.23	11.5	9.8	3.8
5	Galactose infiltrated	28	0.91	1.17	0.25	12.5	10.8	4.8
6	Mannose infiltrated	1	1.60	1.67	0.07	3.5	7.3	1.3
7	Mannose infiltrated	23	1.60	1.93	0.31	15.5	12.5	6.5
8	Mannose infiltrated	28	1.87	2.24	0.35	17.5	17.3	11.3
9	Glucose infiltrated	1	1.60	1.75	0.14	7.0	10.3	4.3
10	Glucose infiltrated	23	1.17	1.63	0.44	22.0	21.0	15.0
11	Glucose infiltrated	28	1.37	1.87	0.48	24.0	24.3	18.3

with different plants. The mutual interconversion of glucose, fructose, and mannose can be accomplished in vitro in very dilute alkaline solution through the LOBRY-BRUYN enolic transformation. Because of the ease with which glucose and fructose are interconverted in plants, it is considered possible that the transformation takes place through this mechanism. The fact that mannose was also found to be converted to glucose and fructose in the barley plant strengthens the view that the interconversion of these three monosaccharides in the plant takes place by the mechanism of enolization, but there is still no experimental evidence to support this assumption. It is even more difficult to account for the transformation of galactose into glucose, since this sugar has no common enolic form with glucose or fructose. The theoretically possible change of position of the fourth hydroxyl group in the glucose molecule by the mechanism of the WALDEN inversion, whereby it could be converted into galactose, also has no experimental proof.

The existence of hexosephosphates in plants shown by several investigators (**1, 7, 13**) in recent years strongly indicates that phosphorus plays an important rôle in the mechanism of sucrose formation. The isolation of a mixture of glucose and fructose-phosphoric acid esters from pea leaves by one of the writers (**7**) suggests that phosphorylation of the glucose and fructose components is probably a necessary step in the synthesis of sucrose in the plant. Support of this view can be found in the work of KURSANOV and KRYUKOVA (**9**). These authors showed that synthesis of sucrose in chicory and sugar beet leaves was always accompanied by an accumulation of phosphoric esters, also the synthesis of esters by sucrose synthesis. Under conditions of phosphate deficiency the capacity of the plant to synthesize sucrose decreased considerably. The introduction of phosphate in such plants restored their normal synthetic capacity. SYSSAKYAN (**12**) has also found that in the case of phosphorus-starved sugar beet leaves, the synthesis of sucrose was inhibited to a considerable extent as compared with normal plants. HANES's (**2**) recent important contribution, that of synthesizing starch from glucose-l-phosphate in the presence of phosphorylase from potato tubers, demonstrates the important rôle of phosphorus in the mechanism of carbohydrate synthesis. It is suggested that in the interconversion of monosaccharides some intermediate phosphorylated compound might also be involved.

Mannitol, sorbitol, and gluconic acid were not utilized by barley to form sucrose. Apparently the plant did not contain any enzymes which could oxidize the sugar alcohols or reduce the sugar acid. The formation of glucose from glyceric aldehyde indicates that sugars may be formed from tricarbon atom compounds containing a reducing group.

Summary

1. Sucrose can be synthesized in barley plants when either of the following monosaccharides are supplied: glucose, fructose, mannose, and galactose. It is therefore evident that the plant possesses a mechanism to convert these monosaccharides into glucose or fructose.

2. Maltose and lactose can also be utilized by barley for sucrose formation. The plants, apparently, possess enzymes which are able to hydrolyze these disaccharides to their respective monosaccharides, which are then used for the synthesis of sucrose.

3. Synthesis of sucrose from monosaccharides can take place in etiolated plants in the dark. The process is therefore independent of light and does not require chlorophyll.

4. Arabinose, xylose, mannitol, sorbitol, and gluconic and pyruvic acids were not utilized by the plant for sucrose formation.

5. When excised barley shoots respired for 24 hours the sucrose gradually diminished, apparently due to hydrolysis, but the reducing sugars remained approximately at a constant level throughout that period. It was only after the sucrose was entirely depleted, that a diminution in the reducing sugars could be observed. It was also shown that an excess of reducing sugars induces synthesis of sucrose, while an excess of sucrose favors the reverse process, hydrolysis.

6. Synthesis of sucrose did not occur without the presence of oxygen, which indicates that this process is aerobic.

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REFRACTIVE INDEX AS AN ESTIMATE OF QUALITY BETWEEN AND WITHIN MUSKMELON FRUITS[1,2]

T. M. Currence and Russell Larson

(WITH TWO FIGURES)

Introduction

Many difficulties arise in connection with testing the eating quality of fruits. Table quality depends on taste to a great extent, and uniform agreement among different individuals as to the desirability of certain flavors seems to be an elusive objective. An evaluation may be obtained by getting numerous estimates in quantitative classes and averaging them. This, however, is laborious and generally too tedious for extensive use. The present work on muskmelons arose in connection with varietal improvement studies with this crop. It was evident that a heterozygous population produced progenies with wide differences in quality that were traceable to quality differences in their parents. Inconsistencies in such observations, however, indicated the desirability of more accurate determinations of quality than was possible by having a few individuals taste the fruits and give them quality ratings. Use of the hand refractometer that is commonly used in rapid testing of sugar beet juice for sugar content appeared to offer a solution to the problem, provided it gave a reliable index to eating quality. This method has been used by different workers (**1, 2**) for testing melon quality but there has not appeared in the literature any information as to the degree of error that may be involved when quality is estimated by this method. The data which follow are intended to show mainly the mathematical relation between such refractometer readings and quality ratings when the latter is determined by a number of organoleptic tests and to compare the variation that was found in such tests with the variation in estimates based on refractometer readings.

Materials and methods

In the initial study, juice of 30 muskmelons was tested by means of a Zeiss hand refractometer, a rapid and simple test for total soluble solids contained in the juice. Whereas the index of refraction of a substance is the ratio of the sine of the angle of incidence to the sine of the angle of refraction, the

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scale of the Zeiss hand refractometer is graduated from 0 to 30 in percentage of dry substance in the solution. The indices of refraction for 0, 5, 10, 15, 20, 25 and 30 per cent. by weight of aqueous sucrose solutions at 20° C. are 1.3330, 1.3403, 1.3479, 1.3557, 1.3639, 1.3723, and 1.3811 respectively.

The strains represented were progenies of a single heterozygous plant with varying amounts of inbreeding and selection covering one to five generations. The pedigree of the original plant is not known, but it is certain from types segregated that honeydew entered into the parentage. The samples of juice for refractometer tests were taken from the central area of the fruits and the fruits were then rated fór quality by 19 people. Five arbitrary classes were set up ranging from one to five, with one being the lowest class for eating quality and five being the highest. Only whole numbers were used by the testers in rating the fruits. The readings obtained were averaged for each of the fruits and this mean was used as the quality rating of the respective melon.

A second set of observations, somewhat similar but differing in certain details, was collected. Ten fruits taken at random from a field of mixed strains were tested by the refractometer, juice being taken from the central area of the fruits. The fruits were quartered by cutting them longitudinally and transversely, the longitudinal cut being made as nearly as possible parallel to the surface of the ground upon which the melon rested as it developed. Figure 1 illustrates the four sections and the identifying numbers. It is obvious that a larger number of fruits would have been desirable but it was thought that 40 samples was near the maximum number that could be properly tested by an individual at one time.

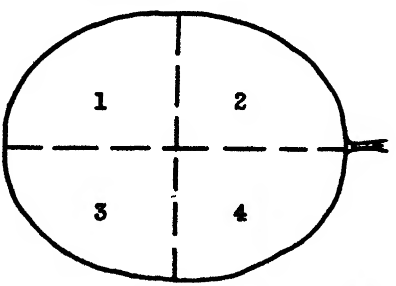

FIG. 1. The cuts made to divide muskmelon fruits into four sections and the respective numbers of the sections.

The 40 sections were each tested by the hand refractometer and laid out at random. Then eighteen persons tested, independently, each of the 40 samples and rated them for quality in classes ranging from one to five. This arrangement provides comparisons and tests of different parts of the fruits which were not possible in the original test.

Results

Data on quality from the initial test

The analysis of variance for the first test is summarized in table I. It is evident that fruits differed significantly in quality, and that the testers differed significantly in rating them. The F value exceeds the 1 per cent. point in both instances. The standard error of 0.930 indicates the high degree of variability that occurs from such a test. The standard error of the mean of 18 tests is $\frac{0.930}{\sqrt{18}}$ or 0.219 which is taken as the standard error of the melon means. A difference of approximately 0.620 between means is statistically significant and one of 0.850 is highly so. Calculations show that ratings by 7 testers would have been needed to establish the statistical significance of a difference of one class between two melons. This emphasizes the relationship of taste variations to measurement of quality differences. The relationship is more emphatic if the calculation is made to find the number of tests needed to establish statistical significance of a difference of 0.5 of a class. It is found that the standard error of the mean must reduce to 0.176 which would require approximately 28 observations.

TABLE I

Analysis of variance of quality ratings on 30 muskmelon fruits

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	Standard error
Melons	29	223.0	7.70	*	
Testers	18	71.0	3.94	*	
Error (melons × testers)	522	452.3	0.866		0.930
Total	569	746.3			

* Exceeds 1 per cent. point.

The refractometer readings as shown in table 6 cover a wide range and undoubtedly represent melons of several distinct classes. The relation of these results to the quality ratings will be discussed more fully under the heading of correlation.

Data on quality from the second test

The possibility that the blossom end of a muskmelon is higher in quality than the stem end is frequently encountered and likewise it is possible that the top and bottom halves of the fruit differ in quality. In order to test these possibilities, the analysis of variance was made as summarized in table II. Since this analysis shows the sections to be significantly different, it is desired to compare the means for differences. Figure 2 A is a diagrammatic presentation of the quality means of the four sections. A difference of

TABLE II

ANALYSIS OF VARIANCE FOR QUALITY SCORES OF FOUR SECTIONS OF EACH OF 10 MUSKMELON FRUITS BASED ON INDIVIDUAL ESTIMATES OF 18 TESTERS

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	Standard error
Testers	17	78.28	4.61	†	....
Melons	9	375.73	41.75	†	....
Sections	3	12.97	4.32	*	....
Melons × sections error (A)	27	37.84	1.40	†	1.18
Testers × melons	153	240.00	1.57	†	....
Error (B)	510	324.18	0.64	......	0.80
Total	719	1069.00	....	......	....

* Exceeds 5 per cent. point.
† Exceeds 1 per cent. point.

approximately 0.211 is significant and one of approximately 0.286 is highly significant. Therefore, although the difference is small, section 2 differs significantly from the other three sections but none of these differ among themselves by statistically significant amounts. It is apparent that quality ratings differed between halves of the fruits. The blossom end was slightly but significantly higher than the stem end, and the bottom half was insignificantly higher than the top half.

The means of the halves have a standard error of 0.062. These are also

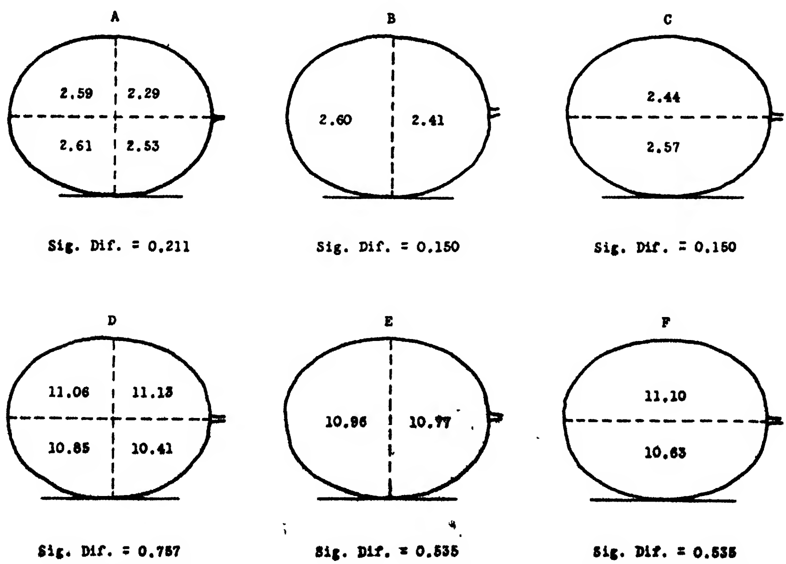

FIG. 2. Diagrammatic presentation of quality ratings A, B, and C and refractometer readings D, E, and F of different areas of muskmelon fruits.

shown diagrammatically in figures 2 B and 2 C. A difference of 0.150 between these is statistically significant and one of 0.202 is highly so. It is apparent that the two ends of the fruit show a difference that is significant. In the case of the upper and lower halves, the difference is smaller and does not reach the five per cent. point. The odds, however, are approximately 12:1 against a difference of this size being the result of random samples. The data definitely seem to suggest minor differences in quality between various parts of the fruits and therefore the desirability of defining areas when the fruits are tested for quality.

The interaction sums of squares, shown in table II, are of some interest. That of testers × melons, being significant, indicates that various testers rated the same melons differently. Stated otherwise, the quality of a melon may appeal to a certain tester but would rate low in the opinion of another. Thereby the point is illustrated that individual estimates vary as to what constitutes desirable or undesirable quality. The detailed results on this interaction are shown in table III. The differences in estimates may be readily seen by observing a typical case such as the ratings given melons A and I by testers 11 and 13.

TABLE III

THE QUALITY RATINGS OF 10 MUSKMELON FRUITS AS DETERMINED BY 18 TESTERS RATING FOUR SAMPLES OF EACH FRUIT

Tester	Melon										Mean
	A	B	C	D	E	F	G	H	I	J	
1	3.50	2.00	4.25	2.00	1.00	3.00	2.00	3.00	1.75	2.75	2.52
2	4.25	2.50	5.00	1.75	1.00	4.00	2.25	2.75	3.00	1.25	2.77
3	3.00	2.25	4.75	2.00	1.00	3.75	3.00	2.75	2.75	3.75	2.90
4	3.00	1.00	3.50	2.75	1.00	2.50	3.00	1.50	2.75	1.75	2.27
5	2.00	1.25	2.00	1.75	1.50	2.00	2.25	2.50	2.50	1.00	1.87
6	1.75	3.00	5.00	2.00	1.25	2.50	2.50	1.50	3.75	1.50	2.47
7	2.50	2.00	3.00	3.00	1.00	2.75	1.75	2.00	3.75	1.25	2.30
8	3.50	2.25	3.25	2.75	1.00	3.00	2.75	2.75	4.50	3.25	2.90
9	3.50	3.00	4.50	2.50	1.25	3.00	2.75	2.00	3.75	3.00	2.92
10	3.50	2.00	4.50	3.00	1.00	3.25	2.25	2.25	3.75	2.75	2.82
11	3.75	1.00	4.00	2.00	1.50	3.50	1.75	2.00	2.75	1.50	2.37
12	2.75	2.75	5.00	3.75	1.25	3.00	3.00	2.50	3.75	3.50	3.12
13	1.25	1.75	4.75	2.50	1.00	2.50	2.75	2.75	4.25	1.25	2.47
14	3.75	1.75	4.50	1.75	1.00	2.75	1.75	2.50	3.25	1.50	2.45
15	2.50	2.75	2.00	3.50	1.00	3.75	2.25	2.00	3.25	1.25	2.42
16	2.75	1.75	3.25	2.00	1.00	2.25	2.50	1.50	3.50	1.50	2.20
17	1.75	1.25	3.25	2.50	1.25	2.00	2.25	2.50	3.50	1.00	2.12
18	1.75	2.50	2.25	2.75	1.00	2.50	2.25	1.50	3.50	1.50	2.15
Mean	2.82	2.04	3.82	2.46	1.11	2.89	2.39	2.24	3.33	1.96	

The melons × sections interaction was significantly greater than that for error (B). Table IV shows the data on this interaction. It is evident that considerable variation occurred in a certain section for different melons.

TABLE IV

QUALITY RATINGS FOR FOUR SECTIONS OF EACH OF 10 MUSKMELON FRUITS AS DETERMINED BY 18 TESTERS PER SAMPLE

MELON	SECTION OF MELON				MEAN
	1	2	3	4	
A	2.78	2.89	2.67	2.94	2.82
B	2.17	1.78	2.39	1.83	2.04
C	4.11	3.33	4.33	3.50	3.82
D	2.11	2.39	2.44	2.89	2.46
E	1.22	1.00	1.05	1.17	1.11
F	3.06	2.67	3.06	2.78	2.89
G	2.72	2.00	2.17	2.67	2.39
H	2.39	1.94	2.22	2.39	2.23
I	2.94	3.06	3.83	3.50	3.33
J	2.44	1.78	1.94	1.67	1.96
Mean	2.59	2.28	2.61	2.53	

The interaction can be visualized by noting that section 1 had the highest rating in three of the fruits, section 2 in none of them, section 3 in three, and section 4 in two fruits. In two cases there was an equal rating between sections for the highest score; one and three in the case of fruit F, and one and four for fruit H. Thus, it is seen that although section 1 had the high score in most cases, section 3 had the highest general average, and that the section ratings differed in different melons.

CONSISTENCY OF EXPERIENCED TESTERS

Considering the variation in estimates by the organoleptic tests the question may arise as to the possibility of reducing this if the individuals making the ratings have had previous experience at it. It may be possible that extensive sampling would enable an individual to be more consistent in his ratings. Three of the samplers in the second test were individuals who had had considerable experience at testing various fruits and vegetables in research work. Attempting to determine the effect of this background on their comparative ratings, correlation coefficients were calculated between the mean melon ratings of each of these three and the mean of each melon for 12 of the other testers. The scores of the three remaining testers were also correlated with the scores of the 12 combined and were found to give essentially the same correlations as that for the experienced testers. It appears in this instance, that the experienced testers were not superior over three testers selected at random in estimating the mean of several tastes.

REFRACTOMETER DATA FROM THE SECOND TEST

When the 40 refractometer readings are divided and analyzed, as shown in table V, it is seen that statistical significance between sections is not indi-

cated although the quality differences shown by figures 2 A, 2 B, and 2 C, are significant. This suggests that the quality score differences for different sec-

TABLE V

ANALYSIS OF VARIANCE FOR 40 REFRACTOMETER READINGS ON FOUR SECTIONS OF EACH OF 10 MELONS

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F	STANDARD ERROR
Melons	9	217.25	24.14	*	
Sections	3	4.24	1.41		
Error	27	18.51	0.68		0.825
Total	39	240.78			

* Exceeds 1 per cent. point.

tions were possibly the result of factors other than soluble solids, as indicated by the refractometer readings. TUCKER (4) has shown that the sugar content varies in different parts of a watermelon fruit. SCOTT and MACGILLIVRAY (3) obtained uniform readings in soluble solids for longitudinal sections of muskmelons but found an increasing gradient from the stem to the blossom end for cross sections. In the present study the odds for some of the comparisons, although not 20:1, are rather high. As example, section 3 compared with section 4 gives odds of 7:1; and the top half compared with the bottom half gives odds of approximately 15:1. It is of interest that these differences are not in the same relationship as the differences between the quality scores. This seems to be further evidence that the quality differences between sections may result from factors other than sugar content. From the above it is understandable that the analysis of covariance failed to show significant correlation between quality score and refractometer readings within fruits although it was quite definite between fruits.

CORRELATION DATA

The correlation coefficient for the first set of data calculated between the refractometer readings and the quality score means shown in table VI is + 0.636 and is highly significant. The regression of quality on refractometer reading is 0.167. This is the average amount by which the quality score varied with a unit change in refractometer reading. Using this regression coefficient and a refractometer reading, it is possible to estimate the quality score. The accuracy of the estimate is indicated by the last column in table VI. The standard error of estimate is 0.493, which may be considered as an average of the differences between observed and estimated quality. This is a decidedly lower value than the standard error of 0.930 shown in table I.

In the second test, having the correlation broken up, it is possible to consider the relationship for within melons and between melons by Fisher's

TABLE VI

REGRESSION OF QUALITY ON REFRACTOMETER READING FOR MUSKMELONS (QUALITY RATING IS THE MEAN AS DETERMINED BY THE ESTIMATES OF 19 INDIVIDUALS GIVING RATINGS OF 1 TO 5 FOR QUALITY)

FRUIT NO.	REFRACTOMETER READINGS	OBSERVED QUALITY	ESTIMATED QUALITY	OBSERVED – ESTIMATED
1	12.0	2.63	3.03	– 0.40
2	11.8	2.37	3.00	– 0.63
3	9.5	1.95	2.60	– 0.65
4	12.8	2.47	3.17	– 0.70
5	8.5	1.58	2.45	– 0.87
6	11.0	2.79	2.87	– 0.08
7	13.4	3.68	3.27	0.41
8	11.2	2.89	2.89	0.00
9	12.0	3.10	3.03	0.07
10	8.0	2.63	2.36	0.27
11	9.2	3.42	2.56	0.86
12	12.5	3.05	3.12	– 0.07
13	13.0	3.26	3.20	0.06
14	7.6	2.74	2.30	0.44
15	8.2	2.11	2.40	– 0.29
16	9.2	3.37	2.56	0.81
17	8.4	3.26	2.33	– 0.93
18	13.4	2.95	3.27	– 0.32
19	10.0	2.89	2.70	0.19
20	6.8	1.79	2.16	– 0.37
21	9.4	2.58	2.60	– 0.02
22	8.0	2.11	2.36	– 0.25
23	14.0	3.37	3.36	0.01
24	9.4	3.05	2.60	0.45
25	12.2	3.16	3.07	0.09
26	9.4	2.58	2.60	– 0.02
27	6.2	2.00	2.06	– 0.06
28	5.0	1.37	1.86	– 0.49
29	7.0	1.95	2.20	– 0.25
30	10.8	3.89	2.83	0.96

analysis of covariance method. A regression coefficient for between melons is arrived at as well as one for the association within melons or between sections. As previously stated, however, it was found that the correlation within fruits was not significant, and the correlation between refractometer reading and quality score mean for between fruits was essentially the same as that for total. Furthermore, the one refractometer reading originally taken on the 10 fruits when correlated with the quality means gave approximately the same value as that when the mean of four refractometer readings was used.

Inasmuch as this single reading approximates the results of the more extensive readings and represents considerable saving in time, it seems desirable to limit further analysis to the one reading data. The correlation coefficient between this value and quality score is +0.862 and is highly significant. The regression of quality on refractometer reading is 0.264 in this instance. The weight in grams of the 10 melons also was correlated with

quality, the value being + 0.655 and slightly above the five per cent. point. This is surprising and the relationship probably is not generally true of muskmelon fruits. Fruit size being a character easily measured, however, it should be more adequately studied in relation to quality between types as well as within uniform strains. The multiple correlation coefficient between the three measurements was found to be + 0.909. As shown by table VII, the standard error of estimate is 0.342. Based on this 10-melon sample, it is apparent that estimating quality from the multiple regression is relatively accurate.

TABLE VII

REGRESSION OF QUALITY SCORE ON FRUIT SIZE AND REFRACTOMETER READINGS FOR MUSKMELON FRUITS (QUALITY RATINGS DETERMINED BY 18 INDIVIDUALS MAKING FOUR TESTS PER FRUIT)

Fruit identification	Weight in grams	Refractometer reading	Observed quality rating	Estimated quality	Difference
A	1490	10.4	2.81	2.22	0.59
B	1350	11.0	2.04	2.33	−0.29
C	3850	13.3	3.82	3.69	0.13
D	1290	12.2	2.45	2.63	−0.18
E	970	6.0	1.11	.90	0.21
F	2000	12.6	2.88	2.95	−0.07
G	2640	11.0	2.38	2.72	−0.34
H	1550	9.6	2.23	2.02	0.21
I	1380	13.8	3.33	3.08	0.25
J	1600	10.9	1.95	2.38	−0.43

Comparing the respective standard errors, it is noted that the above 0.342 is approximately equal to that of 12 testers sampling by tasting, since 1.18 divided by the square root of 11.9 approximately equals 0.342. Thus, it seems that the simple weight and refractometer tests were useful tools for calculating eating quality under the conditions of this test. The calculation for getting the estimated quality is 0.0003 times the weight in grams plus 0.264 times the refractometer reading and this sum minus 0.975. Although the calculation is a simple one, a melon breeder would seldom use it in testing selections. The simple procedure of comparing refractometer readings would suffice in most instances.

Summary and conclusions

Thirty muskmelons representing a mixture of types were tested for the percentage of soluble solids in the juice by means of a hand refractometer. The melons were then rated quantitatively in ascending classes from one to five by 19 people tasting them. Analysis of the data indicates that the melons were significantly variable in quality and that testers varied significantly, some tending to rate the fruits low and others to rate them high. The standard error of 0.930 illustrates the difficulty of satisfactorily

classifying the fruits by tasting unless the mean of a number of opinions is obtained. Ratings by three experienced testers did not approach the mean scores appreciably more closely than the ratings of three testers selected at random.

In a second test, 10 muskmelon fruits were cut into four sections after they were tested by the refractometer. Each section was tested separately by the refractometer and also rated for quality by 18 testers. The results indicate slight but significant differences in quality between different parts of the fruits. The blossom end averaged higher than the stem end. The bottom half was not significantly higher than the upper half. Interaction data show poor agreement among the different individuals in rating the fruits for quality. Also it is shown that certain sections were high in some melons and low in others. Analysis of the refractometer readings do not show statistically significant differences between the different parts of the fruits and such differences as did occur were somewhat opposed to the differences in quality. It is, therefore, suggested that the quality differences between sections may have resulted from factors other than those measured by the refractometer.

For the first set of data, quality rating estimates based on the regression of quality on refractometer readings gave an error of estimate appreciably lower than the standard error by tasting. A correlation coefficient of + 0.636 and a regression coefficient of 0.167 were found. In the second lot of data, there was positive correlation between fruit weight and quality score (+ 0.655) as well as between refractometer reading and quality score (+ 0.862). Using multiple regression the error of estimate was further reduced so that it approximately equals the standard error of the mean of 12 samplers. The coefficient of correlation for the three characters is + 0.909. The information on the relation between fruit size and quality is thought to be incomplete but the extent to which refractometer readings can be used in estimating muskmelon quality is considered well demonstrated by the foregoing material.

THE MINNESOTA AGRICULTURAL EXPERIMENT STATION

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SOME EFFECTS OF SUPPLEMENTARY ILLUMINATION WITH MAZDA LAMPS ON THE CARBOHYDRATE AND THE NITROGEN METABOLISM OF THE ASTER (*CALLISTEPHUS CHINENSIS* VAR. HEART OF FRANCE)

RAYMOND WENGER

(WITH TWO FIGURES)

Introduction

Greenhouse crops show marked responses to supplementary artificial lighting with Mazda lamps (4). The present study was undertaken to determine whether there are differences in composition between groups of asters subjected to various supplementary light intensities and those which did not receive supplementary light; and to further ascertain if these differences in chemical composition, especially carbohydrates and nitrogenous compounds, can be correlated with the responses of the aster. This plant was chosen because it blooms in a long day and is suitable for study in short day.

GARNER and ALLARD (3) found that many of the plants with which they worked might be classified according to the length of day which favors their reproductive processes, namely: (a) short day type, (b) long day type, and (c) indeterminate type. MURNEEK (7), and PARKER and BORTHWICK (10) have shown that flower primordia can be initiated in Biloxi soybeans by subjecting the seedlings to a short day of 7 to 8 hours. After this induction period, the plants grown in a short photoperiod fruited while those grown in a long photoperiod remained vegetative. MURNEEK (7) points out that whether plants are vegetative or reproductive is apparently brought about directly by the length of day. PARKER and BORTHWICK state that changes in chemical composition that can stand in causal relationship to the initiation of flower primordia must occur before initiation can take place.

The work of KRAUS and KRAYBILL (6) has shown that the type of response in the tomato plant is associated with the carbohydrate content in relation to the available nitrogen supply. NIGHTINGALE (8) found that tomatoes grown under short day conditions and with available nitrogen supply produced vegetative growth. Long days with available nitrogen produced vigorously fruiting plants. In a later study (9) it was shown that Salvia, a short day plant, can be thrown into the vegetative state by lengthening the day. Carbohydrates and nitrates accumulate under short day conditions. When the plants are transferred to long day conditions, there is a loss of carbohydrates, and associated with this loss there is a more

rapid assimilation of nitrate and increased vegetative growth. MURNEEK (7) has indicated that as a result of a short photoperiod, seedling soybeans show a rapid accumulation of the storage forms of carbohydrates, especially starch, and a rapid synthesis of nitrogenous substances; these changes, however, probably have nothing to do with initiation of flower buds but rather promote development of the reproductive organs. ECKERSON (2) pointed out that reductase decreases in soybean plants which receive only 8 hours of daylight as compared with those which receive full daylight. It was thought that this might explain the accumulation of nitrates in a short day. HIBBARD (5), however, has found that the reductase activity was only partially inhibited in Biloxi soybeans by a short day, but showed no definite change as the plants responded to the different daylengths.

Methods

Seedlings, 1 to 1.5 inches, were transplanted to 2.5-inch clay pots and allowed to grow about one month before they were transferred to the different plots. The plants were transplanted to larger pots as needed.

In the first, or fall, series of experiments, four long day conditions and one short day condition were studied. All plants received normal daylight. The plants under long day conditions received supplementary illumination from Mazda lamps from 9:00 P.M. to 7:00 A.M. for a period of 10 hours each night; the plants under short day conditions, or controls, received only the normal daylight.

Representative plants were taken at each sampling date to have a minimum of 100 grams for the nitrogen determinations, 50 grams for the carbohydrate determinations, and 15 grams for dry weight studies. The leaves were clipped into small pieces and used for samples.

After the dry weights were obtained these same samples were used for total nitrogen (11). Another sample was thoroughly ground and a water extract made which served for the determination of the total water extractable nitrogen. After aliquots were drawn for the total water extractable nitrogen, the water extractable protein was coagulated by means of acetic acid and heat and filtered off. Aliquots were taken from the filtrate for the water soluble nitrogen. Additional aliquots served for the ammonia and nitrate (13), and amino nitrogen determinations (14). Total protein was obtained by subtracting the water soluble nitrogen from the total nitrogen.

In testing for carbohydrates, the samples were plunged into sufficient 95 per cent. hot ethyl alcohol to bring the alcohol content to 80 per cent; to this had been added 0.5 gram of $CaCO_3$. The alcohol extract was used for determining reducing substances and sucrose (1, 12). Starch was determined on the residue by the taka-diastase and subsequent hydrochloric acid hydrolysis method. The reducing power of the solution was determined as dextrose. These values were converted to starch by multiplying by 0.9.

Results

Experiment I

In a preliminary study[1] of the effect of supplementary light on asters observations were made on fresh weights, stem length, and time of flowering. Chemical analyses were also made on these plants. This work was repeated in more detail in experiments II and III.

Experiment II. first series, 1933

First sampling.—Aster seed was sown in flats August 26, 1933, transplanted to pots September 14, and transferred to supplementary light plots on September 24. The average greenhouse temperature was 55° F. The first sampling was taken on October 24, after 30 days of growth. Results of the analyses are given in table I.

TABLE I

Effect of supplementary illumination on the carbohydrate and nitrogen content of Aster. Expressed as percentage of the fresh weight. October 24, 1933. 30 days

Analyses	Foot candles				
	100	10	1	0.3	Control*
Averages fresh weight per plant in grams	4.17	4.25	3.99	3.58	2.81
Dry matter	12.62	12.35	13.28	13.75	16.24
Reducing substance	0.69	0.65	0.62	0.63	0.70
Sucrose	0.27	0.31	0.33	0.41	0.50
Starch	2.93	2.90	2.81	3.36	4.48
Total carbohydrates†	4.23	4.21	4.23	4.62	6.20
Total nitrogen	0.43	0.47	0.44	0.47	0.60
Soluble nitrogen	0.10	0.10	0.10	0.12	0.21
Protein nitrogen	0.33	0.37	0.34	0.35	0.39
Nitrate nitrogen	0.04	0.05	0.05	0.06	0.08
Amino nitrogen	0.03	0.03	0.04	0.04	0.06

* No supplementary light at night.
† Total carbohydrates are the sum of reducing substances and starch as dextrose, and sucrose as invert sugar.

Second sampling.—This sampling was taken on November 20, after 57 days of growth. The control plants were deep green in color and were low, spreading in appearance. Those plants which received supplementary light were a lighter green. Stems and leaves were analyzed separately. The results of these analyses are given in table II. The reaction of these plants to various degrees of supplementary lighting is shown in figure 1.

As shown in table II there is a progressive increase in fresh weight per plant as the supplementary light intensity increases. These data also show

[1] This was part of a cooperative project of the Horticultural and Agricultural Chemistry Departments, Purdue University Agricultural Experiment Station.

TABLE II

EFFECT OF SUPPLEMENTARY ILLUMINATION ON THE CARBOHYDRATE AND NITROGEN CONTENT OF ASTERS. EXPRESSED IN PERCENTAGE OF THE FRESH WEIGHT. NOVEMBER 20, 1935. 57 DAYS

ANALYSES	FOOT CANDLES				
	100	10	1	0.3	CONTROL*
Averages fresh weight per plant in grams	9.37	8.89	8.22	8.06	3.75
Dry matter	12.2	14.5	18.2	20.0	21.0
Reducing substance	1.34	1.51	1.48	1.44	1.30
Sucrose	0.32	0.42	0.51	0.70	0.71
Starch	1.87	2.44	2.42	2.00	1.90
Total carbohydrates†	3.77	4.68	4.71	4.40	4.16
Total nitrogen	0.31	0.30	0.33	0.30	0.49
Soluble nitrogen	0.05	0.04	0.04	0.06	0.07
Protein nitrogen	0.26	0.26	0.29	0.24	0.42
Nitrate nitrogen	0.03	0.02	0.02	0.03	0.04
Amino nitrogen	0.03	0.03	0.04	0.04	0.06

* No supplementary light at night.

† Total carbohydrates are the sum of reducing substances and starch as dextrose, and sucrose as invert sugar.

an increase in the percentage of total carbohydrates and starch from the control plants of the 10-foot-candle plot. The 100-foot-candle plot shows a decrease in total carbohydrates. The soluble nitrogen, nitrate, and amino nitrogen fractions are low in the lighted plots and high in the control plots.

This appears to indicate that in the control plants the nitrogen fractions are not utilized as rapidly or as efficiently in the formation of proteins and in vegetative extension as they are in the lighted plots.

FOURTH SAMPLING.—This was taken on Jaunary 30, 1934, after a growth

FIG. 1. The effect of supplementary light on the growth of Aster. First series, November 20, 1935. 57 days. (1), 100 foot candles; (2), 10 foot candles; (3), 1 foot candles; (4), 0.3 foot candles; (5), Control.

Plants were taken from plots which received 10 hours additional light each night of the intensities indicated.

period of 128 days. All plots were in bloom but not all of the plants in each plot. The controls were deeper green while the plants that received supple-

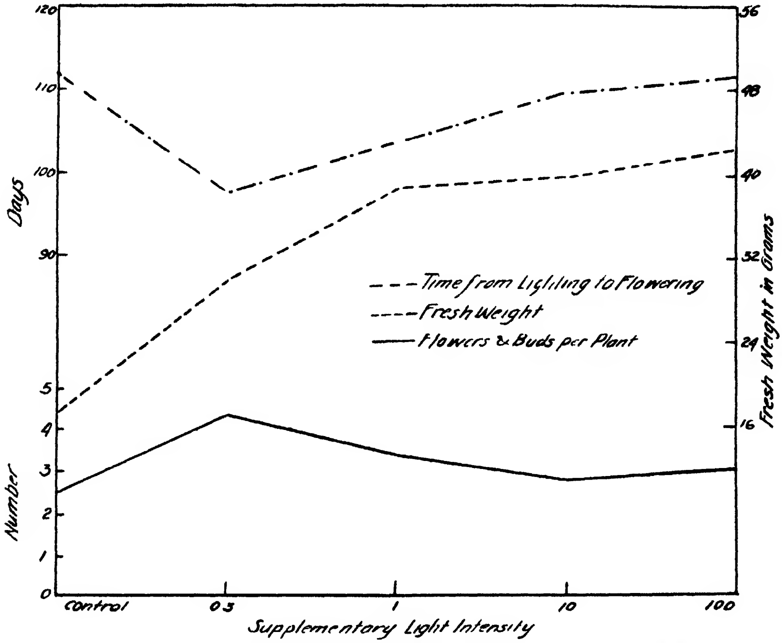

FIG. 2. The effect of supplementary light on time of flowering, fresh weight, and flowers per plant at time of harvest. First series, January 30, 1934. 128 days.

TABLE III

EFFECT OF SUPPLEMENTARY ILLUMINATION ON THE CARBOHYDRATE AND NITROGEN CONTENT OF ASTER. EXPRESSED IN PERCENTAGE OF THE FRESH WEIGHT. JANUARY 30, 1934. 128 DAYS

ANALYSES	FOOT CANDLES				
	100	10	1	0.3	CONTROL*
Average fresh weight per plant in grams	42.6	39.1	39.3	30.0	16.8
Dry matter	15.58	15.76	15.72	15.70	16.84
Reducing substance	0.90	0.88	0.93	1.18	1.27
Sucrose	0.32	0.38	0.38	0.40	0.35
Starch	2.41	2.52	2.27	1.89	2.02
Total carbohydrates†	3.92	4.03	3.87	3.71	3.90
Total nitrogen	0.22	0.20	0.21	0.24	0.39
Soluble nitrogen	0.10	0.07	0.07	0.08	0.15
Protein nitrogen	0.12	0.13	0.14	0.16	0.24
Nitrate nitrogen	0.008	0.01	0.01	0.01	0.01
Amino nitrogen	0.02	0.02	0.02	0.02	0.02

* No supplementary light at night.
† Total carbohydrates are the sum of reducing substances and starch as dextrose, and sucrose as invert sugar.

mentary light were a light green. Data relative to the time of flowering, fresh weights, and flowers per plant are presented in figure 2. Results of analyses are given in table III.

All of the buds were open on the plants in the 0.3-foot-candle plot while the plants in the 100-foot-candle plot had the most unopened buds; the plants in the control plot had the lowest number of unopened buds. Earliest flowering occurred in the 0.3-foot-candle plot.

Differences in composition expressed as percentages of the total carbohydrates and total nitrogen are not as evident in this cutting as in the first two cuttings (tables I, II). The differences in composition of the plants of the various plots diminish as the plants reach the flowering stage.

Experiment III. Second series, 1934

A second series of experiments was begun in February 1934. Only two groups received supplementary light (10 and 0.3 foot candles) for a period of 10 hours each night. A third group (control) received no additional light. Four samples were taken at approximately 30-day intervals. The fresh weight, height of plants, time of flowering, and chemical composition followed the same general trend as those in experiment II.

Discussion

The heights and fresh weights of the lighted (long day) plants increase as the supplementary light intensity increases. Withrow and Benedict (**15**) have shown that asters which receive 0.3 foot candle supplementary light or more have a greater dry weight than the control plants.

Carbohydrates.—As seen in table I the plants show a decrease in the percentage of total carbohydrates, sucrose, and starch as the supplementary light intensity increases. Associated with this decrease in percentage of total carbohydrates there is an increase in fresh and dry weights as compared with the controls. Though carbohydrates are produced in large quantities in the longer day of the lighted plants, they were utilized rapidly for growth and were stored but slowly in these plants. There was an accumulation of carbohydrates in the control plants. The percentage of total carbohydrates is relatively high in the plants of the 0.3-foot-candle plot (table I). It is probable that the removal of the products of photosynthesis was not as rapid as in the plants receiving the higher light intensities. Withrow (**15**) has suggested that the greater dry weight of plants receiving 0.3 foot candle, as compared with controls, may be due to an indirect effect of the supplementary light at night on the photosynthetic activity during the following day, possibly through a faster removal during the night of soluble carbohydrates accumulated during the day.

Nitrogen.—The control plants are higher in the percentage of nitrates

and water-soluble nitrogen in the early stages of growth as compared with the lighted plants. Associated with the lower percentage of nitrate and water-soluble nitrogen in the plants which receive supplementary light there is an increase of fresh weight and vegetative extension.

Summary

1. Asters receiving no supplementary illumination are least vegetative, bloom latest, and have the smallest number of flowers per plant. The plants are high in percentage of carbohydrate and soluble nitrogen but, in the short day length, are unable to utilize these compounds in vegetative extension.

2. Lengthening the day with Mazda lamps results in greater vegetative activity, larger number of flowers per plant, and earlier flowering.

3. Earliest flowering and largest number of flowers per plant occurs with the plants receiving supplementary illumination of the lowest intensity —0.3 foot candle. Such plants are medium in vegetative activity and relatively high in the percentage of carbohydrates and soluble nitrogen during the early stages of growth.

4. As the supplementary light intensity is increased to 100 foot candles vegetative activity increases, and the percentage of carbohydrates and soluble nitrogen decreases in the early stages of growth.

5. Significant differences in composition of the groups of plants occurred only in the early samplings. When the plants reach the flowering stage, the composition is very similar regardless of the treatment. Analyses of plants made at the time of flowering or maturity are of little value in interpreting carbohydrate-nitrogen relationships in growth and reproduction.

The writer is indebted to Dr. H. R. Kraybill for the advice and encouragement given in carrying on this investigation. He also wishes to thank Mr. Robert B. Withrow of the Horticultural Department, Purdue University Agricultural Station, for the installation and permission to use the lighting equipment.

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AN INEXPENSIVE INTEGRATING LIGHT RECORDER[1]

V. G. SPRAGUE AND E. M. WILLIAMS

(WITH THREE FIGURES)

In recent years environmental factors, such as temperature and light, have been receiving increased consideration in experimental procedures. Under greenhouse conditions, the temperature and length of day can be controlled but it is impossible, except by excluding sunlight and supplying artificial light, to control the radiant energy which plants receive during a given growth period. The variation in the incident light during an experiment in the greenhouse or in the field may explain in part the differences between replications of the same experiment conducted at different times. Although it may not be possible to control the incident light available to experimental plants at various times, interpretations of the results obtained might be facilitated if the total amount of light which the plant had received were known. The length of day can be easily obtained from available data for each latitude but daily measurements of the total incident light are rarely obtainable. Instruments which will record the intensity of the natural light from day to day are available, but in order to obtain the total incident light, the area enclosed by the intensity curve must be determined. This is a tedious and time-consuming task if an experiment covers more than a few days, and becomes more difficult and less accurate on days when clouds are intermittent. To overcome the difficulties encountered with such a recording instrument, as well as to reduce the cost of equipment, an integrating light recorder was constructed from a few electrical parts obtainable from a radio dealer.

The principle of operation used in this instrument has been described by ANDERSON (1) who placed a small condenser in series with a phototube and a battery. A neon tube in parallel with the condenser flashed when the voltage across the condenser rose to the breakdown voltage of the tube and went out when the voltage dropped to the extinction voltage. RENTSCHLER (2) modified this method by using a cold cathode relay tube in place of the neon tube. The relay tube supplied sufficient current to operate a mechanical relay and thus a counter mechanism. This instrument has found its main use in measurements of ultra-violet radiation.

The recording instrument herein described requires a power pack for operation with ordinary 115 volts, 60 cycles A.C. supply. If a used radio is

[1] Contribution no. 12, of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Northeastern States and the Electrical Engineering Department of the Pennsylvania State College, State College, Pennsylvania.

available its power pack, including transformer, rectifier tube and socket, choke coil, and condensers may be used. Most of these items will be in usable condition, except perhaps for the electrolytic condensers usually supplied. These, however, can be replaced with superior paper or oil-filled condensers of sufficient capacity at a cost of one to two dollars. If a used radio is not available the items required for the power supply may be purchased separately. The total cost of the power supply should not exceed five dollars ($5.00).

The wiring diagrams for both the power supply and integrating instrument are presented in figures 1 and 2.

In operation, light striking the light-sensitive element in the phototube causes a current to flow, which is very closely proportional to the intensity

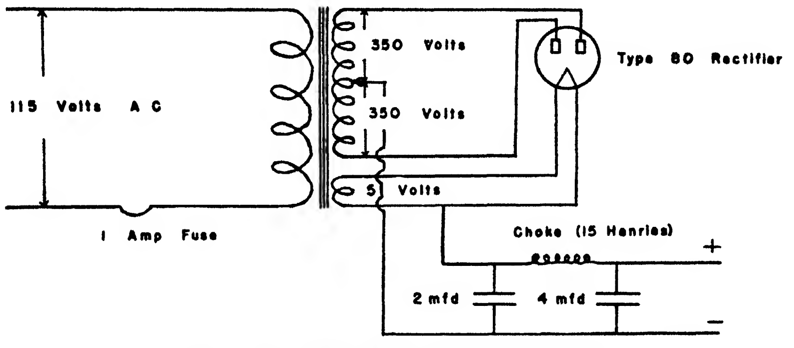

FIG. 1. Wiring diagram for power supply.

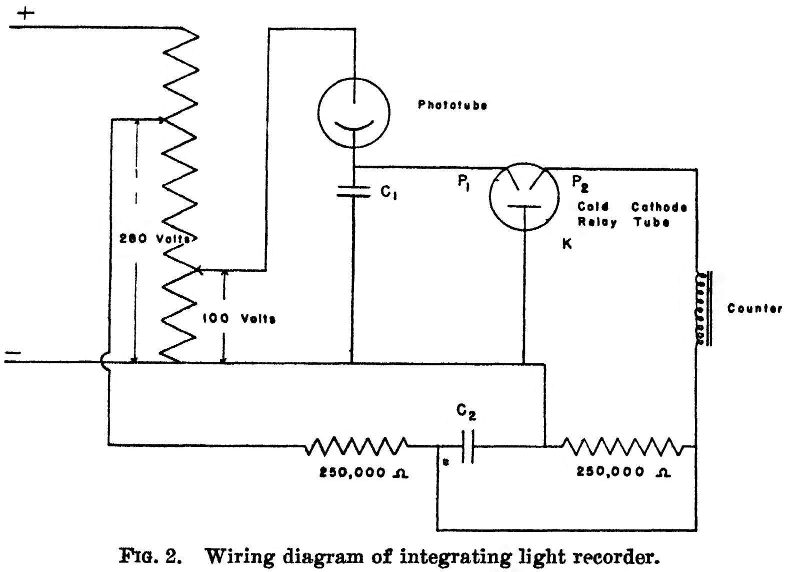

FIG. 2. Wiring diagram of integrating light recorder.

of the light. This current charges the condenser C_1. The relation between current, i (a function of time), and the potential difference, V, at time, t, across the condenser is:

$$V = \int_0^t i\,dt + V_0$$

in which the potential is assumed to be V_0 at time $t = 0$. This potential difference is applied directly between the starting anode (P_1) and the cathode (K) of the cold cathode gas discharge tube (Western Electric type 313C). When the potential, V, reaches a critical value, the gas in the tube ionizes, allowing the condenser C_1 to discharge (at which time the potential returns to V_0) and at the same time discharging C_2. The discharge from this latter condenser passes through a sensitive magnetic telephone relay, pulling down the armature and actuating the counter. As soon as the discharge passes, the tube deionizes and the circuit returns to its initial condition. A very sensitive counter is required because the amount of current passing through the relay tube depends on the capacity of the condenser, C_2, which is limited not only by the current rating of the tube but also by the small allowable time delay in recharging the condenser, C_2, between successive operations of the counter.

A counter which has worked very satisfactorily was constructed from a dollar alarm clock. The balance wheel was removed and a 1/32-inch hole drilled in the escapement lever. One end of a fine brass spring wire was passed through this hole and the other end fastened to the armature of the sensitive telephone relay. The clock and relay were so mounted that the throw of the relay armature moved the escapement lever sufficiently to allow one tooth of the escapement wheel to pass, as would occur in the normal operation of the clock. In this counter, the gear mechanism of the clock is driven by the main spring while the magnetic relay supplies only the small force required to trip the escapement. The clock can be calibrated easily before the balance wheel is removed, by counting the number of escapements per minute in normal operation. With the cheaper clocks this usually is 120. Therefore, with such a counter, 7200 operations would be required for a complete revolution of the minute hand and 86,400 would represent a complete revolution of the hour hand. The regular face of the clock may be removed and a face reading in total number of discharges substituted, or, by proper calibration, the face may be calibrated to read directly in any convenient units.[2]

[2] Light has been defined as the radiant flux within the wave length limits of sight, evaluated according to its capacity to produce visual sensation. The foot-candle is the practical unit of illumination and represents the illumination on a surface, normal to the direction of the light, from a point source of one International Candle, one foot distant. Since the human eye is not equally sensitive to all regions of the visible spectrum and

The capacity of the condenser, C_1, which stores the charge depends on the current output of the phototube. Since there may be considerable difference in the sensitivity of photo-electric cells even of the same type and by the same manufacturer, it is necessary to determine the size of the condenser, C_1, experimentally. An intensity of 500 foot-candles incident on the particular General Electric PJ–22 phototube used resulted in about 50 discharges per minute when a 2.0 mfd. (microfarad) condenser was employed. Less frequent operation of the counter could be obtained by using a larger condenser but error owing to leakage in the condenser would have increased; more frequent operation through the use of a smaller condenser was undesirable owing to the failure of the counter to register accurately at speeds greater than 80 operations per minute. The use of a smaller condenser would be desirable if the instrument is designed to record lower maximum light intensities. The authors have experienced no difficulty in recording light intensities well below one foot candle with condenser capacities of the order of 0.01 mfd.

The instrument is adaptable to a number of uses, inasmuch as the phototube can be placed at any distance from the counter and several counters can be operated from one power supply unit. The maximum intensity striking the phototube should not exceed that recommended by the manufacturer, which in the case of the General Electric PJ–22 tube is 500 foot-candles. In this preliminary arrangement for the measurement of sunlight, the radiation is received on a horizontal surface, similar to Weather Bureau practice. The maximum intensity on the phototube is reduced to 500 foot-candles by means of two sheets of frosted opal glass. WALLACE (3), in a study of methods of sampling visible radiation, has indicated that a single light sensitive unit placed inside and at the base of an opal glass sphere is to be preferred as the receiver for radiation measurements to be used in physiological work. In any receiver care should be exercised to prevent direct sunlight from striking the phototube and to prevent the temperatures of the phototube from exceeding 50° C.

The linearity of the above instrument has been checked from 5 to 450 foot-candles incident on the phototube. A straight line relationship exists between the intensity of the light and the current output of the phototube when the quality of light is unchanged. A decided shift in the quality of light either to the blue or red end of the spectrum alters the current output of any of the phototubes now available. The General Electric PJ–22 tube

since the mechanical equivalent of light varies with the wave length, it is not possible to convert foot-candles into energy units except for monochromatic light or for heterogeneous light of known and constant composition. For many applications, however, comparative data would be acceptable. For this purpose, the integrated light may be expressed in foot-candle hours which is the product of intensity and time.

is least sensitive to light of about 5000 Å wave length and increases in sensitivity to longer and shorter wave lengths (see fig. 3). For measuring

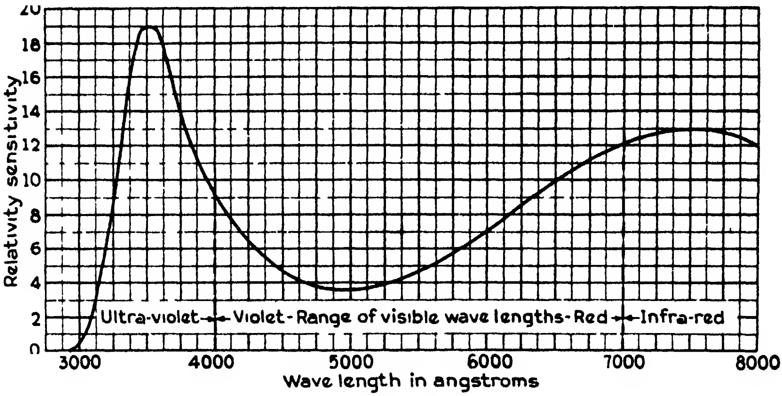

FIG. 3. Static sensitivity characteristic of the PJ–22 phototube. (Reproduced through the courtesy of the General Electric Company, Schenectady, N. Y.).

natural daylight, in which the quality does not vary greatly except at sunrise and sunset and where comparative data are primarily desired, this limitation of the phototube is not serious.

After assembly, the instrument was calibrated against a standardized Weston Model 603-foot-candle meter using sunlight at noon on a clear day as a light source. For comparative data the natural light available during any period may be expressed as foot-candle-hours which is the intensity in foot-candles multiplied by the time in hours. Inasmuch as this instrument is integrating, the average intensity will be available. In calibration of the above instrument it was found that an average sunlight intensity of 240 foot-candles passing through two frosted opal glasses before reaching the phototube produced one counter operation per minute. The number of foot-candle hours during any period is equivalent to the number of discharges divided by the number of discharges the counter would make at an average intensity of one foot-candle for one hour. In this calibration, at one foot-candle, there was 1/240 discharge per minute or 60/240 discharges per hour.

$$\text{Then total f.-c.-hours} = n \div \frac{60}{240} = \frac{n \times 240}{60} = 4n$$

where n = number of discharges during the period.

Example:

16,800 discharges on July 21 $= 4 \times 16{,}800 = 67{,}200$ f.-c.-hr.

Several examples of the data obtained are presented here as an indication of the variation in daylight at State College, Pa., during the months the instrument has been in operation.

Daily values of light available in foot-candle-hours:

April 8,	28,705
April 9,	6,312
April 10,	14,578
April 11,	50,496
April 12,	25,549

Weekly values of light available in foot-candle-hours:

April	1– 7,	inclusive,	280,935
April	8–14,	inclusive,	172,228
April	15–21,	inclusive,	186,515
April	22–28,	inclusive,	245,116

Monthly values of light available in foot-candle-hours:

March	1–31,	inclusive,	678,351
April	1–30,	inclusive,	1,000,754
May	1–31,	inclusive,	1,087,771
June	1–30,	inclusive,	1,201,993
July	1–31,	inclusive,	1,507,462
August	1–31,	inclusive,	1,198,561

After five months of uninterrupted operation a new calibration was made and compared with the original. There was no appreciable change, indicating that in all probability the characteristics of the gas relay tube and the phototube change very slowly with age. The change in calibration with the fluctuations in supply line voltage usually encountered may be expected to be negligible. Operation of the relay tube and the characteristics of the condensers are affected only by larger fluctuations in ambient temperature than those normally encountered in a laboratory or a greenhouse. No great change in sensitivity of the phototube would be expected if the 50° C. maximum ambient temperature recommended by the manufacturer is not exceeded.

The upkeep of the instrument is very small, since no moving electrical contacts are used which might be oxidized, and no mechanical parts other than the magnetic relay armature and counter are employed. The cold cathode relay tube requires no attention and has an almost indefinite life if it is not overloaded. It would seem that the low original cost of the instrument described (less than $25 as compared to commercially available instruments ranging in price from $150 to $500), its low current consumption (about 10 to 15 watts), and its probable long-life would put within the reach of those interested in light as it effects growth, a means of studying some of the factors not heretofore determined.

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PRODUCTION OF SOLUTES IN GROWING EPIDERMAL CELLS

WILLIAM A. BECK

(WITH ONE FIGURE)

Growth by cell enlargement, which is characteristic of plant cells, may be defined as the irreversible increment in the volume without appreciable increment in the mass of the protoplasm. Investigation of this kind of growth was given considerable impetus by URSPRUNG and BLUM (**12**) when they discovered that the turgor of young cells is minimum when the growth is maximum, and also by WENT (**14**) and his school when they proved that the growth-promoting substance which induces this kind of growth originates in the region of cell proliferation.

Although it appears evident that the growth-promoting substance must exercise its effect on the living protoplasm (*cf.* **10**) there is still some question about the effect produced directly or indirectly on the cell wall and the production of solute in the protoplasm which is osmotically active in the cell sap cavity.

KLEBS (**5**) concluded from his experiments that the protoplasm is directly responsible for cell enlargement, which is, of course, hardly tenable in view of the facts known to us at present. HEGLER, SACHS, DEVRIES (**3**, **7**, **13**) and others assumed that cell enlargement is directly due to the stretching of the wall, which itself is caused by the turgor. This view was decidedly disputed by SCHWENDENER and KRABE (**8**), who contended that the wall grows actively by a process of intussusception with the cooperation of the protoplasm. PFEFFER (**6**) explains how the growth of the wall may be the result of active and passive growth, with consequent changes in the elastic constants of the wall. The fact which URSPRUNG and BLUM established beyond a doubt, that the turgor is minimum in the most actively growing cells (**12**) makes it certain that the turgor plays at most a secondary rôle in the process of cell enlargement. It also makes it questionable if the solutes which are necessary to maintain the turgor are elaborated by the protoplasm to a degree which is commensurate with the growth of the cell.

It is the purpose of the present investigation to determine the amount of solute which is produced during the various stages of cell enlargement.

Material and methods

The enlargement of the cells in different phases of development was first determined with the aid of an eye-piece micrometer. Then the osmotic value at incipient plasmolysis and the degree of contraction in passing from the normal state to the state of incipient plasmolysis were determined for

the same phases according to the methods given by URSPRUNG (11). Knowing the volumes in the two states and the concentration at incipient plasmolysis, it became possible to calculate the concentration of the sap in the normal state according to the equation:

$$(1)\ O_n/O_g = V_g/V_n \text{ or}$$
$$(2)\ O_n = O_g \times V_g/V_n;$$

in which O_n is the concentration of the cell sap in the normal state; O_g, the concentration in the state of incipient plasmolysis; V_n, the volume of the cell in the normal state and V_g, the volume in the state of incipient plasmolysis (*cf.* **11**, p. 1111).

Since the amount of solute in a solution varies directly as the volume and directly as the concentration, the total amount of solute present in the normal cells may be expressed in arbitrary units as the product of the normal volume and the normal concentration. In our particular case it was found that the diameter of the cells remains practically constant during cell enlargement, so that it was sufficient to multiply the concentration by the one variable, namely the length, in order to obtain an adequate expression for the total amount of solute present expressed in microns × mol.

The epidermal cells of Helianthus seedlings were employed. As is well known, proliferation of cells occurs only within a very small zone of the hypocotyl (less than 1 mm. long) and then growth occurs by cell enlargement. Since the cells in the neighborhood of the stomatal complex are very irregular they were avoided and relatively long series of regular cells were preferred.

The region from the base of the cotyledons to a point 35 mm. beyond was investigated. For convenience it was divided into 7 zones, each 5 mm. long. Etiolated seedlings that were 90 hours old and from 45 to 50 mm. high, which were raised under controlled conditions (temperature 25° C. 90 per cent. relative humidity) in peat and sand, from seeds of the 1939 harvest of a special strain cultivated by the author for the last five years were employed. The individual differences in the plants were negligible.

The sizes in the cells were determined for three points in each zone, but the average osmotic value at incipient plasmolysis, and the change in volume from the normal state to the state of incipient plasmolysis were calculated as an average for the entire zone and expressed as effective at the mid-point of the zone. Each reading accepted at any point for concentration of the sap or the length of the cell is the average of 30 independent determinations.

Results and discussion

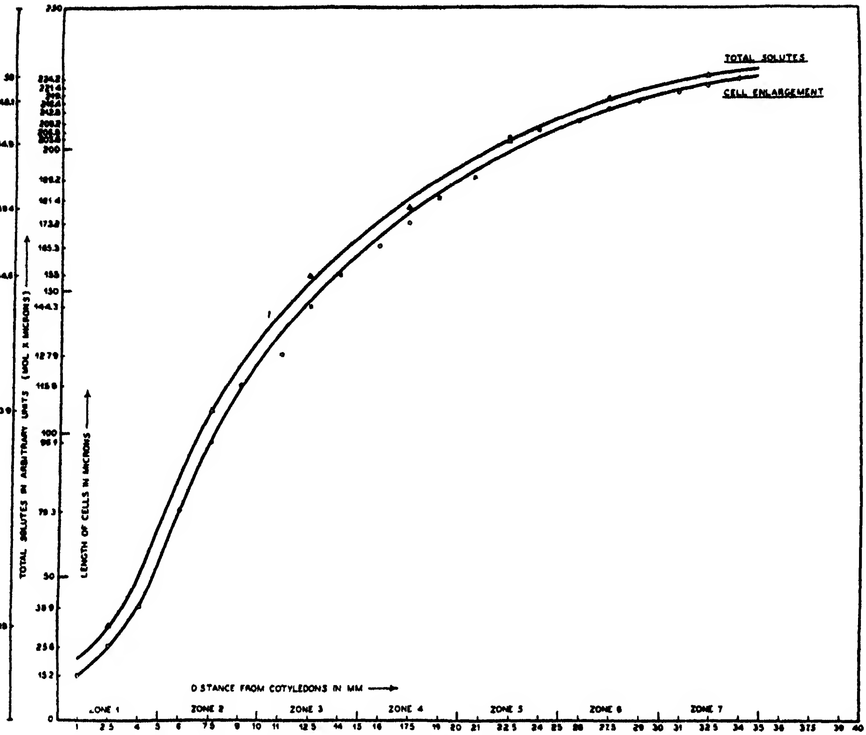
The results obtained are assembled in table I and the graphs (fig. 1) which show the variation in the length of the cells and the variation in the total amount of solute present in the cells as they grow older make the comparison of the results easy.

TABLE I

THE SIZE OF GROWING EPIDERMAL CELLS OF *Helianthus annuus* SEEDLINGS AND THE AMOUNT OF SOLUTE WHICH THEY CONTAIN

ZONE	DISTANCE FROM THE COTYLEDONS	AVERAGE LENGTH OF THE CELLS	AVERAGE EQUIVALENT CONCENTRATION OF THE SAP	TOTAL SOLUTE PRESENT IN ARBITRARY UNITS
	mm.	*microns*	*mol.* ($C_{12}H_{22}O_{11}$)	*microns* × *mol.*
	1.0	15.2		
1	2.5	25.6	0.270	7.18
	4.0	38.9		
	6.0	79.3		
2	7.5	96.1	0.248	23.90
	9.0	115.8		
	11.0	127.9		
3	12.5	144.3	0.243	34.60
	14.0	155.0		
	16.0	165.3		
4	17.5	173.2	0.228	39.60
	19.0	181.4		
	21.0	189.2		
5	22.5	203.6	0.225	44.90
	24.0	206.8		
	26.0	209.2		
6	27.5	212.8	0.226	48.00
	29.0	216.4		
	31.0	219.0		
7	32.5	221.4	0.226	50.00
	34.0	224.2		

The fact that the curves run almost parallel shows that the production of solute must be practically proportional to the increment in volume or to the growth by cell enlargement. Both curves are typical growth curves. They show an inflexion at a point corresponding to the point on the hypocotyl which is 6.5 mm. from the base of the cotyledons. This is evidently the point of maximum growth and production of solute. These results are in agreement with those published elsewhere (*cf.* **1**) regarding the relative growth in the hypocotyl of seedlings which were strictly similar to the ones used in the present work. There it was shown that the most favorable zone for studying growth by cell enlargement is the zone between 4.65 and 6.4 mm. from the base of the cotyledons. There a measuring microscope was used and the hypocotyl was intact except for the markings. In the present experiment zone 3 still shows considerable growth and solute production, but in zone 4 a decided decrement of both rates is noted and in zone 7 the rates of both are almost 0. In other experiments which still await publication, employing strictly similar plants, the suction tension and the wall pressure and the turgor were determined for the same regions and it was



CELL ENLARGEMENT IN THE EPIDERMIS OF THE HELIANTHUS HYPOCOTYLS AND TOTAL SOLUTES OF THE CELL SAP CAVITY

FIG. 1. Relationship between cell enlargement and total solutes of the cell sap.

found that the suction tension is highest in the growing region and lowest in the mature cells; the turgor and the wall pressure, however, were least in the growing region and began to increase decidedly in the mid-region of the fifth zone and were maximum in the mature cells where the suction tension was least. It is interesting to note that solute production declines with the increment of turgor and wall pressure, since it shows the increment in wall pressure must be caused by changes in the nature of the wall and not by increment in the production of solutes. Likewise the decline in the suction tension can hardly be attributed to considerable decrement in the rate of the production of solute but rather to increased counter pressure by the wall, which apparently has become more rigid. On the basis of this explanation it can be stated that the walls have become more rigid at a point 22.5 mm. from the base of the cotyledons. This result is in good agreement with that given elsewhere (2) regarding the critical point of the bending of hypocotyls in strictly similar plants, in response to gravity. There it was found that the regions which are farther removed than 25 mm. from the

base of the cotyledons hardly respond at all, and it was explained that the wall of the cells must be more rigid than in the younger cells.

These results are in agreement with the findings of URSPRUNG and BLUM (**12**) referred to above. They are also in agreement with the finding of SÖDING (**9**) who showed that the first phase of growth is irreversible and that the stretching of the wall by turgor must follow this phase, and that the wall must grow by intussusception. These results are furthermore in agreement with those of STRUGGER (**10**) who showed that growth by cell enlargement can hardly be attributed to the growth of the wall alone. He showed that, in the epidermal cells of Helianthus seedlings, the protoplasm is significantly more viscous in the younger cells than in the mature cells and proved the existence of a "protoplasmatic gradient."

Together with the results obtained by URSPRUNG and BLUM (**12**), SÖDING (**9**), STRUGGER (**10**), and HEYN (**4**), the results of this research make it apparent that the growth of the cell by cell enlargement is a physiological process, and that it cannot justly be attributed to the turgor alone, nor to the growth by intussusception in the wall alone, nor to the changes in the state of the protoplasm alone. The production of solutes and the increment in the mass of the wall, and the physical changes in the protoplasm must be regarded as phases of the growth process, or possibly only as concomitant circumstances; but any one alone is not the prime cause of growth.

Conclusions

From the given results it may be concluded that:

1. The production of solute in the growing epidermal cells of etiolated Helianthus seedlings is proportional to the growth of the cells.

2. The production of solute does not increase the turgor of the cells in proportion to the amount of solute produced, and it cannot be regarded as the primary cause of growth.

3. The production of solute certainly aids in maintaining the suction tension of the growing cells and thus insures sufficient water supply for the growing parts of the seedling. It also aids in maintaining sufficient turgor so that the young plastic wall does not collapse, and that growth in the wall by intussusception is facilitated.

Summary

A method is devised and described for determining the amount of solute produced in growing cells.

The enlargement of growing epidermal cells and the amount of solute produced in them are determined and correlated for etiolated Helianthus seedlings.

It is found that the amount of solute produced is proportional to the growth of the cells.

It is concluded that the production of solute facilitates the growth process in general, and the growth of the wall by intussusception in particular, but that it cannot legitimately be regarded as the prime cause of growth by cell enlargement.

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AUTOMATIC CONDUCTIVITY MEASUREMENTS OF CO_2[1]

D. G. CLARK, JOHN SHAFER, JR., AND O. F. CURTIS

(WITH TWO FIGURES)

In research on photosynthesis it is often desirable or sometimes necessary to record automatically frequent measurements of the amount of carbon dioxide absorbed by a leaf or plant. By none of the older methods could carbon dioxide intake be determined over intervals shorter than one or two hours. The measuring of carbon dioxide by its effect on the conductivity of a sodium hydroxide solution is accurate and rapid, but has not previously been made automatic. The excellent spectrometric method of McALISTER

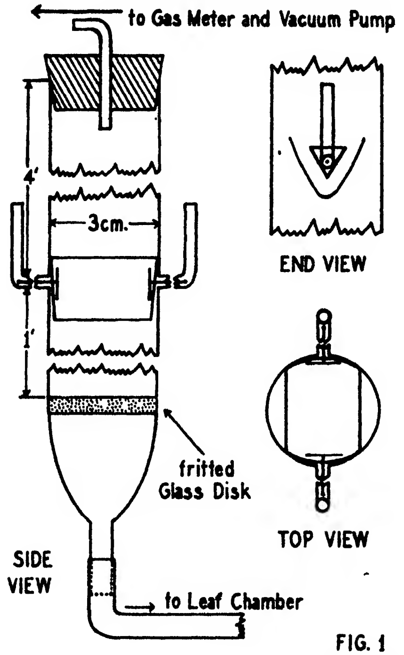

FIG. 1. Equipment for absorption of CO_2.

(2) could be made automatic, but the cost of the basic equipment is considerable. The method to be described in the present note seems to fill the need for rapid automatic recordings. It is based on the conductivity method employed by many other workers.

The arrangement of equipment can best be described under two headings, *viz.*, that used for gas flow and absorption and that for carbon dioxide measurements. The equipment involved in the absorption of carbon dioxide is

[1] The authors are indebted to Dr. A. DOUNCE of the Biochemistry Department at Cornell University and to the Laboratory Division of the Corning Glass Company, for aid in the construction of the absorption tower.

diagrammed in figure 1. The absorption tower[1] in figure 1 needs special description. This is an adaptation of the tower described by HEINICKE and HOFFMAN (1); its basal end contains a fritted glass disk which breaks up the air stream into small bubbles to insure complete absorption of the carbon dioxide. The bubbles rise through the sodium hydroxide solution, exerting a stirring action. Triangular platinum electrodes are fused in the tower a short distance below the level acquired by the liquid surface after the flow of air through the tower has attained equilibrium. The bubbles are diverted from the electrodes by a glass trough and pass to the top of the liquid, where they break. Part of the liquid draining back down the tower is caught in the trough and replaces that which has drained out at the bottom corners. If the trough is properly made the space between the electrodes will be filled with constantly changing, bubble-free sodium hydroxide solution. The solution in the trough does not change in composition as rapidly as that at the bottom of the tower, but the lag is essentially constant and, therefore, immaterial. Since the electrodes are separated by clear liquid, conductivity can be measured at any time, or even continuously. Consequently, automatic records are possible. This is in contrast to the usual conductivity techniques, which involve cessation of air flow and thorough mixing of the absorbing solution before conductivity measurements are made. With that sort of technique frequent automatic readings are impossible.

Since temperature has a marked effect on conductivity, the absorbing solution (NaOH) is kept in a controlled temperature room throughout, except for the brief period while it is being measured in an automatic pipette. By suitable manipulation of several stopcocks the sodium hydroxide solution is measured out and introduced into the absorption tower. Then air is drawn over the leaf, through the absorbent, and through the gas meter.

Naturally, the electrical circuits for automatically recording carbon dioxide should be designed to fit the particular requirements of the operator. The arrangement used by the authors (fig. 2) consists of three separate circuits, though two are in parallel with each other. One circuit contains the absorption and conductivity tower, wired as one arm of a Wheatstone bridge. The other three arms are built with fixed resistances. Accompanying the change from sodium hydroxide to sodium carbonate as carbon dioxide is absorbed, there is a corresponding decrease in the conductivity of the solution. If the initial sodium hydroxide solution is always of the same strength, and if such factors as temperature, electric current and the electrode system remain constant, then the degree of unbalance of the bridge is dependent on the amount of carbon dioxide which has been absorbed by the time of any particular reading. Such being the case, the amount of carbon dioxide absorbed can be measured by its effect on the amount of current going through the recording ammeter connecting the arms of the

Wheatstone bridge. It is believed that quicker readings are obtained by measuring the current across an unbalanced bridge than by balancing the bridge and recording the necessary resistance changes. It is believed, also, that the two methods give readings which are of about the same order of accuracy. In the present arrangement the current is recorded on an Esterline-Angus 5-mil recording ammeter. From experimental data, graphs can be constructed for converting the amperage values to carbon dioxide values. The current passed through the Wheatstone bridge must be of constant voltage. We are using 110-volt 60-cycle A.C. line current regulated by a Raytheon voltage regulator, with satisfactory results.

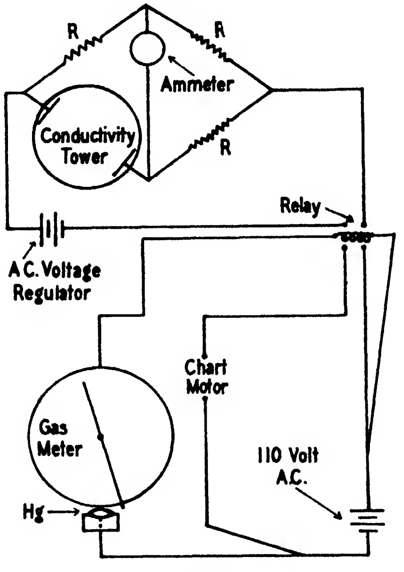

FIG. 2

FIG. 2. Wiring diagram for automatic measuring system.

The "chart motor" is the electric motor that winds the chart on the recording ammeter, and is an integral part of that instrument. It is wired in such a way as to run only while a recording is being made.

The third circuit is the "starter" for the other two. The indicator needle of the wet-test gas meter is extended to dip into a puddle of mercury at a given point of the needle's revolution, thus completing the circuit through the relay coil. The relay closes, thereby completing the other two circuits, and causing the conductivity of the tower solution to be recorded. By varying the number of arms on the meter needle, contact can be made after each complete revolution or after any desired fraction of a revolution. The conductivity record is, therefore, automatically a record of air volume. By having a constant rate of air flow, and by timing that rate at the start of a run, the readings can be converted to time intervals if desired.

At least two towers must be operated simultaneously, since carbon dioxide absorption or loss must be determined by difference. Several towers can be wired into the same electric circuit. The individual recordings require only three or four seconds each. The limiting factors as concerns the number of towers which can be used are: (1) the number of readings per revolution of gas meters; (2) the uniformity of the rate of air flow through the various meters. Unless all meters are running at identical rates, the recordings of two will sometimes coincide. This makes both readings uninterpretable. Obviously, the more recordings there are on the same ammeter in a given time, the more often will two readings coincide.

There are several sources of error which must be considered. Minor fluctuations in the mixing of the solution in a tower will produce minor variations in readings. If a different concentration of carbon dioxide is suddenly introduced, a short period (about 2 minutes) must be allowed for the establishment of the required new mixing gradient in the tower. In our experience, the most serious error is, unfortunately, not controllable: occasionally, the results obtained from one tower will not check with those obtained with replicate towers. As yet, no reason for such inconsistencies has been deduced. As a result of them no single run can be trusted; one must repeat the same experiment a number of different times. The standard deviation of the mean for an individual experiment is about ± 5 per cent. of the mean, when approximately 0.03 per cent. CO_2 is being absorbed.

Summary

There has been described a conductivity system for measuring photosynthesis. Readings are automatically recorded as frequently as desired. As used by the authors recordings are made every two minutes with an air flow of about 50 liters per hour. To achieve accuracy in conjunction with such rapid readings, very dilute hydroxide is being used (0.0013 N). This becomes exhausted in about 20 minutes when normal air is used. However, by changing the strength of hydroxide and the fixed resistances in the Wheatstone bridge, a longer run could be obtained with less frequent recordings. The outfit described should be easily adjustable to give a wide choice of frequencies of readings ranging from one or two minutes up to an hour or more, and should be adaptable to many different experiments.

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A HIGHLY SIMPLIFIED THERMIONIC CONTROL OF TEMPERATURE

Raymond H. Wallace and Ralph J. Bushnell

(WITH TWO FIGURES)

Three recent articles (**1, 2, 3**) describe methods of heat control which seem unnecessarily complicated in view of the present technical development of control tubes in the radio industry. Those methods, just mentioned, use mechanical relays and have wiring schemes which, although not complicated to those acquainted with radio, are nevertheless too difficult for many people to construct. One of the simplest and most effective schemes involving the use of thyratron tubes and no mechanical relays was described by Schwenk and Noble (**4**) in 1937. The scheme here described is a further simplification and adaptation of thyratron methods.

Recently two well-controlled incubators were needed and were obtained by converting two old ovens that had long been around the laboratory unused. These ovens had been controlled by the usual mechanical thermostat and allowed a temperature variation of $\pm 2°$ C. The conversion of the ovens to valuable incubators was done as follows:[1] A five-volt transformer was mounted on top of the oven with the primary connected to the 110-volt line and the secondary connected to the two large prongs of a four-prong socket as filament supply for the control tube. Then the two heavy wires (the 110-volt line for the heater) that had led to the mechanical thermostat were disconnected and one of them soldered to the two small prongs (cathode and shield grid) of the four-prong socket. The other wire was soldered to a clip to *attach to the tip* (*anode*) *of the control tube.* The tube is now wired with plate and filament voltage and all that remains is to connect to the new thermostat. Any type of thermostat can be used. For the ovens just mentioned, coiled bimetallic thermostats were taken out of ten-cent store thermometers. The thermostat was left mounted on the metallic dial of the thermometer, but the hand was broken off and a short shaft soldered in its place. An insulated contact was mounted so the free end of the thermostat could touch it. The thermostat was then mounted inside the oven with the shaft sticking through the top of the oven. An ordinary dial was attached to the shaft. Adjustment for any range is obtained by turning this dial which winds up or unwinds the bimetallic thermostat. The wiring is completed by attaching one contact of the thermostat directly to the control grid, while the other one is connected to the anode through a 10-megohm resistance.

[1] Full-sized blue prints giving all details of construction are available for 25 cents.

It takes about four hours to convert an oven, and the chamber one obtains will maintain temperature to $\pm 0.1°$ C. as measured by mercury thermometers and to $\pm 0.25°$ C. as measured by a recording potentiometer and thermocouple. To get these good values it is, of course, necessary to circulate the air with a small fan.

The tube used in this scheme is the General Electric mercury vapor thyratron, F.G. 95, which carries 2.5 amperes at 1000 volts, but differs from the tube used by SCHWENK and NOBLE (4) in that it has a 5-volt, 4.5-ampere filament and is designed to activate on extremely small grid currents. The tube is so constructed that it will not fire until a plus charge is put on the control grid. When the grid is connected to the anode by the way of the thermostat and grid leak as described above, the tube fires on each plus cycle of the A.C. current and the chamber heater is in action.

The tube maintains a delicate control of the heater, which is very desirable. When the thermostat is well off balance, the tube activates strongly and the heater may draw its full amperage. On nearing its balance, however, the current drops sharply and hovers almost continuously around 0.05 to 0.1 amperes. The tube functions as a variable resistance and consequently the heater never gets hot and is never cold. This eliminates the "all-on," "all-off" condition which is always present in mechanical relays. It likewise makes unnecessary the light-bulb shunt across the heater circuit which SCHWENK and NOBLE used. Measurements with a recorder and a thermocouple showed the floor temperature is as constant as the air temperature being maintained, and is only 1° C. above it. This constant temperature of the heater under continuous load is the most important characteristic of this scheme of temperature control. It makes possible the construction of chambers with little or no stratification even without mechanical stirring. To do this one needs only to embed low-wattage heaters in the six walls of the chamber and connect them all in series with the control tube. The control is obvious at all times by the flicking of the tube, and its continuous glow when the full current is flowing.

It is often necessary in controlling temperature to activate the heater, the reverse of that above, with bimetallic thermostats. Thus, with mercury thermostats, one wants the heater to cut out when contact is made. This can be done very simply with the scheme used here. In figures 1 and 2, the solid lines show the wiring thus used with bimetallic thermostats. If one moves the thermostat from position A and places it in position B, that is, if it is moved from being in series between the anode and the control grid, and is placed in series between cathode and control grid, the tube activates while the thermostat is open but ceases to fire when it is closed. In making this change it will be noted that the 10-megohm leak remains in place between anode and grid while a 500,000-ohm grid leak is added along

with the thermostat between the grid and the cathode. When the thermostat is closed the grid is at the potential of the cathode and the tube does not fire; when the thermostat is open, the grid is at the potential of the anode and the tube fires on each plus half of the cycle as before.

One of us (5) has used the F.G. 95 as control tube in recording potentiometers since 1934, and found them very dependable. Although some of them have had more than three years continuous use, none has burned out in service. They are fragile and, therefore, frequently damaged in shipment. If, however, they function after being heated up a few times, they

FIG. 2

FIG. 1

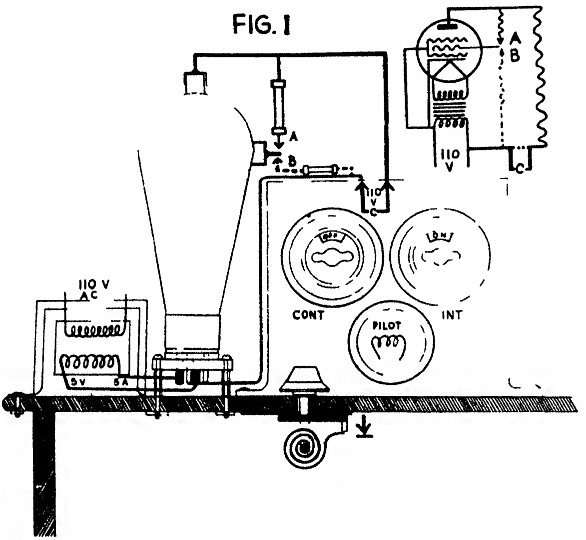

FIG. 1. Pictorial diagram showing connections and spacing of parts; A, position of thermoregulator when it is a bimetallic thermostat; B, position of thermoregulator when it is a mercury thermostat. When A is used, B is left entirely out; but when B is used, the grid leak at A is connected to grid. C is position of usual mechanical thermostat and, if connection is made at the arrow points, the wiring of switches and pilot bulb remain unchanged.

FIG. 2. Schematic diagram showing complete wiring. A, B, and C, the same as in figure 1.

are undamaged and give no trouble subsequently. The total cost of material for converting an oven is less than $15.00; $12.00 for the tube, and $2.00 for the transformer.

The tube activates on from 1 to 1/10 microamperes, so no appreciable current passes through the thermostat contacts. This small current necessitates good insulation in the grid circuit for otherwise, leakages may activate the tube.

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BRIEF PAPER

SPECTROSCOPIC ANALYSIS OF PLANT EXTRACTS FOR CHLOROPHYLLS *a* AND *b*[1]

C. L. COMAR AND F. P. ZSCHEILE

Absorption coefficients for chlorophylls *a* and *b* in ether solution have been determined recently on preparations of high purity.[2] The quantitative application of absorption data to simple plant extracts is the ultimate criterion both for the identity of the standard chlorophyll components with those in the extracts and for the accuracy of the standard absorption coefficients.

It is now possible to analyze plant tissues satisfactorily for total chlorophyll and for percentage of components *a* and *b*. The method is as follows: A weighed sample (about 10 gm.) of fresh leaf tissue is disintegrated in a Waring Blendor in the presence of acetone to which a small amount of calcium carbonate has been added. The resulting mixture is filtered by suction and the pigments are transferred to diethyl ether (Dupont technical, allowed to stand over solid NaOH and distilled). All the acetone is removed from the ether by repeated scrubbing through distilled water and the final solution is dried with sodium sulphate. If care is taken, emulsion formation can be avoided and quantitative results readily obtained. The absorption measurements are made at 6600 and 6425 Å. The spectral region isolated should not exceed 30 Å and large amounts of scattered radiation must be avoided. Other wavelengths may be used when the carotenoid content is not too high.

The absorption coefficients listed above are averages obtained from several pure chlorophyll preparations. Chlorophylls *a* and *b* may be determined most satisfactorily at wavelengths 6600 and 6425 Å. Total chlorophyll may be determined at intersection points of the absorption curves for components *a* and *b*. Component composition may be determined also at wavelengths 6130, 5890, and 5460 Å.

Sample 1 was a spinach extract from which all carotenoids had been removed by washing with methanol, followed by precipitation of the chlorophylls and final removal of carotenoids by washing with petroleum ether.[2] Samples 2 and 3 were aliquots of sample 1 to which pure *beta*-carotene had been added. In these three samples, the *a* to *b* ratio does not represent the natural ratio in the leaf because of preferential loss of *b* during the methanol

[1] Supported by the Herman Frasch Foundation for research in agricultural chemistry, paper no. 213.

[2] ZSCHEILE, F. P., and COMAR, C. L. Bot. Gaz. 102: 463–481. 1941.

TABLE I

ANALYSIS FOR TOTAL CHLOROPHYLL AND PERCENTAGE COMPOSITION

Wave-length	Specific absorption coefficients a	Specific absorption coefficients b	Sample 1 Chlorophyll $a+b$ (carotenoids removed)	Sample 2 Chlorophyll $a+b$ +2 mg. carotene per 100 ml.	Sample 3 Chlorophyll $a+b$ +6.2 mg. carotene per 100 ml.	Sample 4 Spinach leaf extract
6600	102.0	4.5	* 80.3 per cent. a	79.4 per cent. a	79.2 per cent. a	71.1 per cent. a
6425	16.3	57.5	0.0606 gm./l.	0.0590 gm./l.	0.0597 gm./l.	0.0418 gm./l.
6000†	9.95	9.95	0.0600	0.0604	0.0623	0.0422
5810†	8.05	8.05	0.0602	0.0603	0.0622	0.0418
5680†	7.11	7.11	0.0610	0.0618	0.0665	0.0414
6130‡	15.6	8.05	79.8 per cent. a	80.1 per cent. a	83.4 per cent. a	70.4 per cent. a
5890‡	5.90	10.3	78.9 per cent.	77.3 per cent.	72.7 per cent.	66.6 per cent.
5460‡	3.03	6.24	78.2 per cent.	64.8 per cent.	38.6 per cent.	64.5 per cent.

* These values calculated from simultaneous equations.

† Crossing points on absorption curves.

‡ Calculated from absorption at this wavelength and average of values of total concentration which were unaffected by the presence of carotenoids.

washing. Sample 4 was an extract made by the analytical procedure described in paragraph 2.

The following conclusions are evident from the analytical results:

1. All wavelengths listed give correct results when carotenoids are absent.
2. Analytical values at 5890 and 5460 Å are extremely sensitive to the presence of carotenoids, due to the nature of the curves. Even at 6000 Å, carotenoids may cause appreciable error, as shown by sample 3, which has an extremely high carotene content. Note that at 6600 and 6425 Å, the values of sample 3 agree with those of sample 1.
3. The wavelengths, other than 6600 and 6425 Å, that can be used depend upon the carotenoid content of the leaf.

This analysis of spinach is representative of many similar determinations. Broccoli tips and leaves of corn, sweet potato, barley, and tomato have also been analyzed satisfactorily. A more detailed discussion of the chemical precautions and instrumental conditions essential for quantitative results will be presented in another paper.

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PHOTOSYNTHESIS WITH RADIOACTIVE CARBON, AND THE DISTRIBUTION OF THE INTERMEDIATE PRODUCTS IN THE PLANT CELL

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Recent experiments with radioactive carbon (C^{11}) have shown that green plants take up CO_2 reversibly in the dark (2, 3, 5). The evidence accumulated thus far indicates the presence of high molecular weight compounds which bind the CO_2 in a combination stable in boiling concentrated hydrochloric acid.

In this note experiments are described in which the distribution of the initial photosynthetic products within Nitella cells have been investigated. Radioactive carbon was used as a tracer according to the technique described by RUBEN, KAMEN, and HASSID (3).

Experimentation

Nitella plants were used in this work, since these plants release their cell contents very readily on slight crushing. Since no grinding was necessary, it was possible to obtain a maximum of intact chloroplasts by crushing the cells in a 0.5-M glucose solution (1). The cytoplasm and vacuolar sap were separated from the chloroplasts and other bodies by centrifuging. Microscopic investigation of the chloroplasts showed that the majority of them were left intact after this treatment.

1. Intact Nitella plants were exposed to $\overset{*}{C}O_2$ for 25 minutes both in the light and in the dark. At the end of this period the plants were removed from the reaction flasks and immediately crushed in 0.5-M glucose. The cell materials were then separated as stated and their activity tested. Each sample was boiled with 12-N hydrochloric acid, in order to expel any $+4\ \overset{*}{C}$ present. After several washings of the cell wall material with water and of the chloroplasts with 0.5-M glucose, no activity was found either in the chloroplasts or the cell wall material from the plants which were kept in the dark during the exposure to $\overset{*}{C}O_2$. The aqueous, colorless solution obtained from centrifuging of the crushed plant material contained 90 per cent. of the total activity after the first centrifugation, two further washings of the residue removed almost all of the activity from the water-insoluble material. In contrast, the plants which concurrently were kept in the light during this period contained nearly four times as much activity in their chloroplasts as in the non-chloroplast material. This activity in the chloroplasts could not be removed by further washings with 0.5-M glucose.

2. In another series of experiments, Nitella plants were crushed immediately before exposure to $\overset{*}{C}O_2$. This material was filtered through cheese cloth, transferred to Warburg vessels and exposed to $\overset{*}{C}O_2$ for 25 minutes in the dark and in the light. No uptake of $\overset{*}{C}O_2$ was observed either by the cytoplasm and vacuolar sap or by the chloroplasts in a form resistant to boiling hydrochloric acid.

3. Intact Nitella cells were exposed to $\overset{*}{C}O_2$ in the dark for 25 minutes and then crushed also in the dark in 0.5-M glucose. Intact cells were filtered out. The suspension was exposed to light for 20 minutes. No decrease in the activity of the aqueous fraction was observed and no activity was found in the chloroplasts.

Thus it appears that intact plant cells are a prerequisite not only for this particular dark reduction of CO_2 but also for the further reduction of the carboxyl group formed in the dark (**4**). Whatever compound reduces CO_2, whether in the light or in the dark, it seems evident that it is non-chloroplastic, or only weakly adsorbed on the chloroplasts and easily removed by physical injury to the living cell.

The writer is indebted to Dr. S. RUBEN and Dr. M. D. KAMEN for the supply of radioactive CO_2, as well as for their advice and criticism, and to Dr. G. MACKINNEY for his helpful suggestions.

DEPARTMENT OF BOTANY
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NOTES

Annual Election.—The eighteenth annual election of the American Society of Plant Physiologists was held during May and June, 1941. The secretary-treasurer, Dr. W. E. LOOMIS, Department of Botany, The Iowa State College, has announced the results of the election as follows:

President, Dr. Edwin C. Miller, Kansas State College.
Vice-president, Dr. W. E. Loomis, Iowa State College.
Secretary-treasurer, Dr. P. J. Kramer, Duke University.
Executive committee, Dr. W. F. Loehwing, University of Iowa.
Editorial committee, Dr. Otis F. Curtis, Cornell University.

The new officers become active on July 1, 1941. The Dallas meeting will be the most important immediate concern of these officials. They desire, and will need, hearty cooperation in planning and executing the necessary work incident to the eighteenth annual meeting and program. Early replies with titles, and aid wherever it is requested are always appreciated during this busiest season of the year.

Reports of other matters voted upon in the 1941 election will be made in the October number of PLANT PHYSIOLOGY.

Purdue Section.—The Purdue Section of the American Society of Plant Physiologists reports a very successful season during the year 1940–1941 under the chairmanship of Dr. R. E. GIRTON, of the School of Science, Purdue University. Fifteen regular meetings were held during the year, with an average attendance of 25. Two of these were dinner meetings. Dr. F. G. GUSTAFSON, Botany Department, The University of Michigan, addressed the Section at its first dinner meeting in October, on the subject: *Hormones in Relation to Plant Growth.* At the last dinner meeting of the year in May, Mr. GLENN M. SMITH of the Purdue University Agricultural Experiment Station presented his important work on *Sweet Corn Breeding.*

The new officers elected by the Purdue Section for the year 1941–1942 are: Chairman, Dr. F. P. ZSCHEILE, of the Department of Agricultural Chemistry, School of Agriculture; and secretary-treasurer, Dr. W. R. MULLISON, of the Department of Biology, School of Science.

Vacation.—The editor-in-chief will be out of residence from Chicago during the entire summer, until about September 15. Correspondents will please note that, because of rapid movement from place to place, the editor will find it difficult to answer communications promptly. Papers offered for publication should be sent to the office of the secretary of the editorial committee, Dr. W. F. LOEHWING, Iowa City, Iowa, as usual, and other matters allowed to wait until September.

Winthrop John Vanleuven Osterhout.—It is with a great deal of pleasure that we are able to present in this number two fine portraits of Dr. OSTERHOUT, to whom this number of PLANT PHYSIOLOGY is dedicated in honor of his approaching 70th birthday anniversary, which occurs on August 2, 1941. A brief biography was published in PLANT PHYSIOLOGY for January, 1940, at the time he was elected to a CHARLES REID BARNES life membership in the American Society of Plant Physiologists. On behalf of all members of the Society, we extend our best wishes and congratulations to him for the happy occasion of his anniversary, and for a long period of pleasurable activity following his retirement from formal service. He has earned it by nearly fifty years of pioneering work which has advanced our understanding of many physiological processes.

Minor Elements.—The second annual supplement to the third edition of the *Bibliography of References to the Literature on the Minor Elements and their Relation to Plant and Animal Nutrition* has been issued by the Chilean Nitrate Educational Bureau, HERBERT C. BREWER, Director, 120 Broadway, New York. It contains 67 pages, in two-column format, with three indexes, one by elements, one by botanical common names, and one by authors. About 50 elements are included, and 150 crop plants. Slightly less than 400 authors are listed. The annotated bibliography is extremely valuable, and all students of plant nutrition will be grateful for the continuation of this service to research. Members of the Society should obtain a copy of the supplement by writing to Mr. BREWER.

Hunger Signs in Crops.—Interest in the problems of plant nutrition, intimately bound up with animal nutrition, has grown rapidly in recent years. The American Society of Agronomy, in cooperation with the National Fertilizer Association, has published a symposium on *Hunger Signs in Crops.* It is a very attractive monograph, with about 80 plates in colors, and a few less than 100 text figures. The chapters deal with crops, or groups of crop plants, with titles and authors as follows: Why do plants starve? by GEORGE D. SCARSETH and ROBERT M. SALTER; plant-nutrient deficiency in tobacco, by J. E. MCMURTREY, JR.; deficiency symptoms of corn and small grains, by GEORGE N. HOFFER; plant-nutrient deficiency symptoms in the potato, by H. A. JONES and B. E. BROWN; plant-nutrient deficiency symptoms in cotton, by H. P. COOPER; plant-nutrient deficiencies in vegetable or truck-crop plants, by J. J. SKINNER; nutrient-deficiency symptoms in deciduous fruits, by O. W. DAVIDSON; plant-nutrient deficiency symptoms in legumes, by E. E. DETURK; and symptoms of citrus malnutrition, by A. F. CAMP, H. D. CHAPMAN, GEORGE M. BAHRT, and E. R. PARKER.

It is a very valuable survey because it brings a large section of our

knowledge of mineral deficiencies into a single work. Agricultural agents, teachers, and extension men will find it particularly helpful. And many research men will find it handy to use in comparing their own observations. It is written in popular style, but does not sacrifice accuracy in bringing the information to a popular level.

It is printed by Judd and Detweiler, Washington, D. C., at the modest price of $2.50 per copy. The press work is admirable. We recommend this book as one which should be in the hands of all plant physiologists.

Enzyme Research.—The great work by BAMANN and MYRBÄCK, *Die Methoden der Fermentforschung,* continues to come from the press of Georg Thieme, Leipzig. Lieferung 6 contains 336 pages, and Lieferung 7, 416 pages, ending on page 2588. Lieferung 6 continues with the hydrolases, specifically, with the carbohydrases, some of which were presented in Lieferung 5. The phyto- and zoo-amylases, fructanase, glucanases, cytases, and polyuronidases complete the list of carbohydrases. Then follow the nucleases, amidases, proteases, thrombase and blood coagulation enzymes, and oxynitrilese finish out the great group of hydrolases.

The last part of Lieferung 6 begins consideration of the desmolases and enzymes of biological oxidation and reduction. The first part of this section takes up alcoholic fermentation, normal fermentation by living yeasts and zymase preparations.

Lieferung 7 continues with the enzymes of oxydoreduction, glycolytic enzymes, dehydrases, cytochromes, and the oxytropic dehydrases. Section II of the desmolases is a short chapter on the oxyhydrases, such as glucoseoxyhydrase, ascorbic acid oxydase, dioxymaleic acid oxydase, oxalooxydase, amino acid oxydases, and aminooxyhydrases (tyraminoxydase, adrenalin oxydase, aminoxydase, histaminase, and diaminoxydase).

Following this section, the enzymes of aerobic respiration are taken up, the true oxydases. The fourth section takes up the enzymes that make the first attack upon the sugar molecule (hexosephosphorylase); section V, the true desmolases (aldolase, carboxylase, amino acid decarboxylases, etc.); section VI, enolase; and section VII, the hydratases and related enzymes.

Each section is written by some expert in the special field of which he writes, or thoroughly familiar with the progress in methods of attack. It is impossible in a short review to do justice to this outstanding compilation. It deserves a place in the libraries of all institutions where enzyme research is in progress.

The prices stated for these two Lieferungen are: Lieferung 6, RM 25.20; Lieferung 7, RM 31.20. These are the Auslands price quoted by the publisher. It should be remembered that, for obvious reasons, the work is sold only in complete sets.

Modern Fruit Production.—An excellent work by two outstanding horticulturists, Dr. J. H. GOURLEY, and Dr. FREEMAN S. HOWLETT, both of the Ohio Agricultural Experiment Station, and The Ohio State University. The publishers are the Macmillan Co., New York, and the price quoted is $4.50 per copy. It is a book of 579 pages.

There are 17 chapters, several of which are introductory. The story of the fruit industry is told in the first chapter, and the fruit plant and its parts are described in the second. The subsequent chapters are given the following titles: Factors affecting flower formation; site and soil for the fruit plantation; laying out and planting the orchard; cultural practices; fertilizers and manures for the orchard; water relations of fruit plants; pruning of fruit plants; fruit setting; fruit thinning and alternate bearing; the handling and storage of fruit; winter injury; nutrient deficiencies and disorders; propagation and stocks; the origin and improvement of fruits; and orchard, vineyard, and small fruit costs.

The volume represents a complete rewriting and expansion of the previously published *Textbook of Pomology*. It is intended as a practical guide to fruit production, but has included enough of the modern results of botanical and physiological research to make it an up-to-date guide. It is a very clear presentation, and will be a welcome addition to the literature of horticulture.

Annual Review of Physiology.—The third Annual Review of Physiology has been issued by Annual Reviews, Inc., Stanford University, California. It contains 26 reviews, as follows: The relation of bioelectric potentials to cell functioning, by G. H. BISHOP; the physiological effects of radiant energy, by H. LAURENS; physiological aspects of genetics, by A. H. STURTEVANT; developmental physiology, by E. WITSCHI; growth, by C. E. PALMER and A. CIOCCO; temperature regulation, by J. C. SCOTT and H. C. BAZETT; energy metabolism, by T. M. CARPENTER; respiration, by F. C. SCHMIDT and J. H. COMROE, Jr.; physical properties of protoplasm, by E. F. ADOLPH; muscle, by W. O. FENN; the digestive system, by J. E. THOMAS; liver and bile, by W. B. HAWKINS; formed elements of the blood, by G. M. HIGGINS; heart, by C. J. WIGGERS and H. D. GREEN; peripheral circulation, by V. E. HALL; electrical activity of the brain, by H. H. JASPER; the autonomic nervous system, by D. SHEEHAN; the special senses, hearing, visual receptors, and vibratory sensations and pain, by E. BARANYM, R. GRANIT, and Y. ZOTTERMAN, respectively; physiological psychology, by H. S. LIDDELL; kidney, by L. LEITER; metabolic functions of the endocrine glands, by S. SOSKIN; endocrine aspects of the physiology of reproduction, by O. RIDDLE; reproduction in mammals, by M. H. FRIEDMAN; bacterial chemotherapy, by E. K. MARSHALL, Jr.; histamine and anaphylaxis, by W. FELDBERG; and exercise, by A. H. STEINHAUS.

These reviews have been prepared with great care. They portray the recent advances in physiology with clarity and accuracy, and are of the utmost importance to intelligent research in the field of physiology. The price of this volume is $5.00, as usual, and there is no better bargain, anywhere, in the field of physiological literature. The war in Europe has interfered with the receipt of foreign journals, and with the preparation of reviews by foreign experts. Nevertheless, many of the reviews are critical summaries, guiding the reader in appraisal of the progress, others a synoptic review of what has been accomplished. Naturally one prefers the reviews which offer appraisal and criticisms of the investigations, since this is the essence of progress, but all will be found valuable to the working physiologists.

The American Physiological Society, Annual Reviews, Inc., the capable editors, Dr. JAMES MURRAY LUCK and Dr. VICTOR E. HALL, and the collaborators who performed the valuable services in preparing the reviews deserve, and will receive, the thanks and enduring gratitude of all workers who benefit from their efforts. Those who desire to own a copy, should address Annual Reviews, Inc., Stanford University, California.

ALEXANDER PIERCE ANDERSON
NOVEMBER 22, 1862

THIS NUMBER OF PLANT PHYSIOLOGY

IS DEDICATED TO

ALEXANDER PIERCE ANDERSON

IN CELEBRATION OF

HIS EIGHTIETH BIRTHDAY

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OCTOBER, 1941

INFLUENCE OF NITROGEN SUPPLY ON THE RATE OF MULTIPLICATION OF TOBACCO-MOSAIC VIRUS

ERNEST L. SPENCER

(WITH THREE FIGURES)

Introduction

In a former paper the writer (10) reported experiments which showed that the virus activity of juice expressed from tobacco plants diseased with tobacco mosaic was correlated with the amount of nitrogen supplied to the plants. No conclusions could be drawn from these experiments as to whether this increased activity was due to an increase in the rate of virus multiplication or to some other factor, such as greater accumulation of virus particles over a longer period of time, less destruction of virus, or greater dispersion of aggregates of virus particles. Further studies have now been completed which suggest that the variation in virus activity resulting from a change in nitrogen supply may be brought about by a change in the rate of virus multiplication. Results obtained from several repetitions of the experiments to be described below agree closely with those reported in this paper.

Materials and methods

Seedlings of Turkish tobacco (*Nicotiana tabacum* L.), used as experimental host plants, were grown in flats of sand and transplanted at the 3-leaf stage into 4-inch porous clay pots filled with washed white quartz sand. The pots were placed in porous saucers uniformly spaced on a raised bench in a greenhouse, the temperature of which varied between 70° and 80° F. Each seedling received 100 ml. of nutrient solution every other day and was supplied with water whenever necessary between nutrient applications. Low, medium, and high levels of nitrogen were maintained by the use of nutrient solutions, the composition of which has been described previously (10). As in the earlier work, the solutions were all supplemented with boron and manganese, as H_3BO_3 and $MnSO_4 \cdot 2H_2O$, respectively, at a concentration equivalent to 0.5 p.p.m. of each. Plants that received the

high-nitrogen solution (2000 p.p.m. of nitrogen) were retarded in growth, but showed no other external signs of injury. Those that received the medium-nitrogen solution (200 p.p.m. of nitrogen) were comparable in growth with those grown in a rich composted soil. Those that received the low-nitrogen solution (10 p.p.m. of nitrogen) made little or no growth. The plants will be designated for convenience as high-nitrogen, medium-nitrogen, and low-nitrogen plants.

The seedlings were inoculated usually 3 or 4 days after transplanting. One leaf of each plant was rubbed several times with a cheesecloth pad moistened with freshly expressed juice from a tobacco plant diseased with ordinary tobacco-mosaic virus [*Marmor tabaci* H. (**3**)]. Systemic symptoms appeared on all plants about 5 days later. When harvested, plants were minced in a food chopper and the juice pressed from the pulp by means of a small hand press. The expressed juice was cleared of extraneous insoluble material by low-speed centrifugation. In all determinations of virus activity, the cleared juice was used while fresh unless otherwise stated.

Assay for virus activity

The expression "virus content" as used in this paper refers only to the virus activity of expressed juice. There is no reason to suppose, however, that the virus content of the plant is not well represented by the data obtained on samples of expressed juice. *Phascolus vulgaris* L. var. Early Golden Cluster was used as the test plant as suggested by Price (**6**). Bean plants, 9 or 10 days old, were selected for uniformity in the size of their primary leaves, the only leaves used for inoculation. On dark and cloudy days, supplementary illumination from 1000-watt Mazda bulbs was supplied to prevent the bean plants from becoming spindly. The assay for virus activity was carried out as follows: Suitable dilutions of the cleared juice were made with 0.1 M potassium phosphate buffer at pH 7.0. The virus activity of each dilution was then measured by the local-lesion method (**2**). Full use was made of the half-leaf method (**9**) by comparing pairs of treatments on opposite leaf halves in all possible combinations. Since no more than 4 treatments were compared at any one time, it was possible to test each treatment at least once on every bean plant. When half of the test plants had been inoculated, the order in which the samples were applied was reversed. The leaves were syringed with water immediately following inoculation. Each dilution of every sample was rubbed on the upper surface of 36 half leaves.

Although at present there is no satisfactory chemical, physical, or biological measure of the absolute amount of active virus in a solution, it is possible to calculate with a reasonable degree of accuracy the relative virus activity of samples tested at any one time. A comparison of the number of

lesions produced on bean leaves by any definite dilution of extracts shows which sample is most concentrated at that particular dilution but does not show the magnitude of the difference in concentration, because the number of lesions produced is not directly proportional to the dilution of virus used for inoculation. SAMUEL and BALD (9) found that the logarithm of the numbers of lesions produced by tobacco-mosaic virus is a linear function of the logarithm of the virus concentration over a considerable range in concentration. The slope of the straight line fitted to data over this range is of the order of 0.6 but varies, depending upon experimental and environmental conditions. By the use of 3 suitable dilutions of each virus sample, it is possible to determine the slope of the straight portion of the curve and thus obtain a mathematical comparison of the relative virus activity of the samples (**10, 12**). Experimental data on the accuracy of this mathematical procedure will soon be published (**12**). This method of calculation was used in the present study.

Results

RESPONSE IN YOUNG PLANTS

It was anticipated that the age of plants at the time different nutrient treatments were first started might influence the results. Consequently, tests were made with both young and old plants. The first tests with young plants were carried out in May and included a group grown in composted soil. Differential nutrient treatment was started on the day of inoculation. At intervals of 5, 6, 7, 8, and 10 days after inoculation, representative plants were harvested from each of the 4 groups (low-nitrogen, medium-nitrogen, high-nitrogen, and soil-grown plants). The relative virus content in the expressed juice from each set of plants was determined; the experimental data are presented in table I. In this table it is not possible to compare the activity data of one harvest with those of another harvest, because the virus-activity tests were carried out each day on fresh tissue immediately after harvesting. The relative virus content per plant was calculated by multiplying the virus content per milliliter of juice by the average number of milliliters expressed per plant. At each harvest, the control plants grown in composted soil were the largest, although by the 10th day after inoculation the medium-nitrogen plants were nearly equal in size. The high-nitrogen plants were slightly smaller than the medium-nitrogen plants at all times.

The relative virus content of the plants on the various treatments is expressed diagrammatically in figure 1. At all harvests except the first the virus content of the high-nitrogen plants was greater than that of the medium-nitrogen plants. On the 5th and 6th days after inoculation the virus content of the high-nitrogen plants was less than that of plants grown

TABLE I

VIRUS ACTIVITY OF JUICE EXPRESSED AT INTERVALS AFTER INOCULATION FROM DISEASED TOBACCO PLANTS RECEIVING DIFFERENT AMOUNTS OF NITROGEN

NITROGEN LEVEL*	TIME OF HARVEST: DAYS AFTER INOCULATION	NO. OF PLANTS HARVESTED	AVERAGE PER PLANT		NUMBER OF LESIONS ON 36 HALF LEAVES AT A DILUTION OF†			RELATIVE VIRUS CONTENT PER	
			GREEN WEIGHT	VOLUME OF JUICE EXPRESSED	10^{-2}	$10^{-2.5}$	10^{-3}	ML.	PLANT
	days		*gm.*	*ml.*					
Low	5	45	1.2	0.5	2111	1009	458	38	10
Medium	5	40	2.0	1.1	3026	2531	805	103	60
High	5	35	2.0	0.9	3211	2420	834	113	54
Control	5	25	3.4	1.9	2495	2202	934	100	100
Low	6	40	1.6	0.7	793	275	111	20	5
Medium	6	30	2.6	1.3	1681	631	299	72	30
High	6	30	2.6	1.3	2000	982	568	133	56
Control	6	20	5.5	3.1	1631	830	471	100	100
Low	7	30	2.0	0.9	1025	650	434	30	12
Medium	7	20	3.0	1.4	1238	1174	619	113	72
High	7	20	2.8	1.4	1292	1330	731	164	104
Control	7	15	4.8	2.2	1115	1219	606	100	100
Low	8	25	2.6	1.2	2940	1930	1357	189	73
Medium	8	20	4.5	2.1	2954	1772	1509	191	129
High	8	20	4.3	2.1	3450	2060	1289	216	146
Control	8	15	5.8	3.1	2239	1494	1265	100	100
Low	10	25	2.6	1.2	4566	2797	2336	98	33
Medium	10	15	6.4	3.1	6588	3335	3657	295	254
High	10	15	5.5	2.5	6405	3985	3847	428	297
Control	10	12	7.3	3.6	4529	2773	2443	100	100

* The plants used as controls were grown in composted soil.

† At first harvest 5 days after inoculation, the juice was tested at dilutions 10^{-1}, $10^{-1.5}$, and 10^{-2}.

in soil, but on successive days after the 6th day it was progressively greater than that of the soil-grown plants. By the 10th day the high-nitrogen plants had 3 times more virus in their expressed juice than did the soil-grown plants. The virus activity of the medium-nitrogen plants was intermediate between the activities of the plants on these 2 treatments in both harvests after the 7th day. The virus activity of the low-nitrogen plants was considerably less than that of any other plants at all harvests.

This evidence indicates that the addition of a large supply of nitrogen brought about a decided increase in the rate of virus multiplication, the increase being detectable as early as the 5th day after inoculation. Inasmuch as the increased nitrogen supply did not produce any noticeable increase in the rate of plant growth within the experimental period, differential growth cannot be considered as the factor responsible for this change in the rate of virus multiplication. The presence of readily available nitrogen in the sand cultures was influential in increasing virus multiplication at a rate faster than that which occurred in plants grown in composted soil.

In order to determine whether the effect of an increased nitrogen supply might also be detectable by a change in the amount of virus protein in the expressed juice, an experiment of the same general plan as that of the previous experiment was conducted. It was necessary to modify the experimental procedure to some extent, however, to allow for the chemical determination of the virus protein. In order to remove all normal, high molecular weight protein present in diseased as well as in healthy tobacco plants, the plants were frozen at $-14°$ C. immediately after harvesting. It has been pointed out by BAWDEN and PIRIE (1) that the virus protein is unaffected by freezing, whereas the normal protein of corresponding size is denatured and rendered insoluble by such treatment. The plants were held at this temperature in covered dishes for at least 24 hours and then minced. Following treatment of the macerated pulp with 3 per cent. by weight of K_2HPO_4 to decrease the hydrogen-ion concentration to about pH

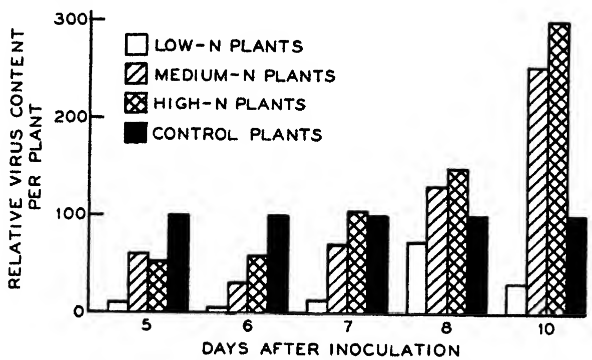

FIG. 1. The relative virus content in juice expressed at intervals following inoculation from plants receiving different amounts of nitrogen.

7, the pulp was allowed to thaw at room temperature. The expressed juice was cleared of insoluble materials by low-speed centrifugation. One aliquot of the clarified juice was analyzed for virus-protein content, another for total-protein content, and a third was assayed for virus activity.

The virus protein was isolated by means of an air-driven ultracentrifuge (**13, 14**) as follows: Ten-ml. aliquots of the clarified juice were ultracentrifuged for 1 hour, at a speed of about 33,000 r.p.m., corresponding to a field with a mean force of approximately 60,000 times gravity. The pellet containing the virus protein was suspended in not over 10 ml. of buffer (0.1 M potassium phosphate at pH 7.0). This suspension was then spun in an angle centrifuge for 10 minutes to remove pigment and aggregated colloidal matter. The supernatant solution containing the soluble virus protein was again ultracentrifuged, and this procedure followed by re-suspension of the virus protein and low-speed angle centrifugation to remove any remaining insoluble material. The supernatant solution was then made up

to its original volume with buffer and analyzed for nitrogen (**11**) by digestion with concentrated H_2SO_4, in the presence of K_2SO_4 and SeOCl, and distillation of the nitrogen present as ammonia.

TABLE II

VIRUS ACTIVITY AND VIRUS-PROTEIN CONTENT OF JUICE EXPRESSED AT INTERVALS AFTER INOCULATION FROM DISEASED TOBACCO PLANTS RECEIVING DIFFERENT AMOUNTS OF NITROGEN

Nitrogen level	Time of harvest: days after inoculation	No. of plants harvested	Average per plant		Relative virus content per		Protein content per ml. of juice (mg. N × 6)	
			Green weight	Volume of juice expressed	Ml.	Plant	Total	Virus
	days		*gm.*	*ml.*			*mg.*	*mg.*
Low	4	25	1.5	0.7	34	20	2.28	0.10
Medium	4	20	2.3	1.2	100	100	3.12	0.10
High	4	22	2.0	1.1	166	152	3.12	0.10
Low	5	25	1.5	0.7	44	24	3.12	0.16
Medium	5	20	2.3	1.3	100	100	3.84	0.28
High	5	22	2.7	1.7	113	148	3.48	0.25
Low	6	25	1.5	0.9	35	19	2.46	0.28
Medium	6	18	2.7	1.7	100	100	6.00	0.60
High	6	18	3.1	1.9	101	113	5.52	0.76
Low	7	25	1.8	1.1	35	14	2.82	0.41
Medium	7	15	4.1	2.7	100	100	6.42	1.05
High	7	15	3.6	2.2	99	81	5.40	1.10
Low	8	22	2.0	1.2	25	11	2.52	0.64
Medium	8	12	4.4	2.7	100	100	7.86	1.68
High	8	15	4.7	2.9	122	131	8.04	2.16
Low	10	20	2.6	1.6	31	14	2.70	0.90
Medium	10	10	5.6	3.5	100	100	8.52	2.04
High	10	10	7.0	4.6	132	173	8.40	2.46
Low	12	20	2.3	1.3	39	9	3.60	1.20
Medium	12	10	8.5	5.7	100	100	9.30	2.76
High	12	10	9.0	6.1	155	166	10.92	3.66

The data recorded in table II are from this experiment carried out in June. Representative plants were harvested from each of the 3 treatments in sand (low-nitrogen, medium-nitrogen, and high-nitrogen) at 4-, 5-, 6-, 7-, 8-, 10-, and 12-day intervals following inoculation. From the virus activity data as recorded in columns 6 and 7, it is apparent that during the period when systemic symptoms of the disease were first appearing, 5 to 7 days following inoculation, the juice from the low-nitrogen plants was only about 35 per cent. as active on the unit-volume basis as was that from plants receiving larger amounts of nitrogen. By the 8th day, it was less than 25 per cent. as active. When calculated on the plant basis, the data indicate

that by the 8th day following inoculation the juice from the high-nitrogen plants contained 12 times more virus than that from the low-nitrogen plants, even though the high-nitrogen plants were only 2 to 3 times as large.

From the data pertaining to the total protein measurements, it is apparent that the protein content per ml. of juice expressed from the low-nitrogen plants increased only 50 per cent. in the 8 days between the 4th and 12th days after inoculation. The plants of the other 2 nitrogen treatments, however, increased about 300 per cent. during the same period. During the 8-day period, the total protein content in the juice from the entire plant increased only 3-fold in the case of the low-nitrogen plants but increased about 14 times in the case of the medium-nitrogen plants and over 18 times in the case of the high-nitrogen plants.

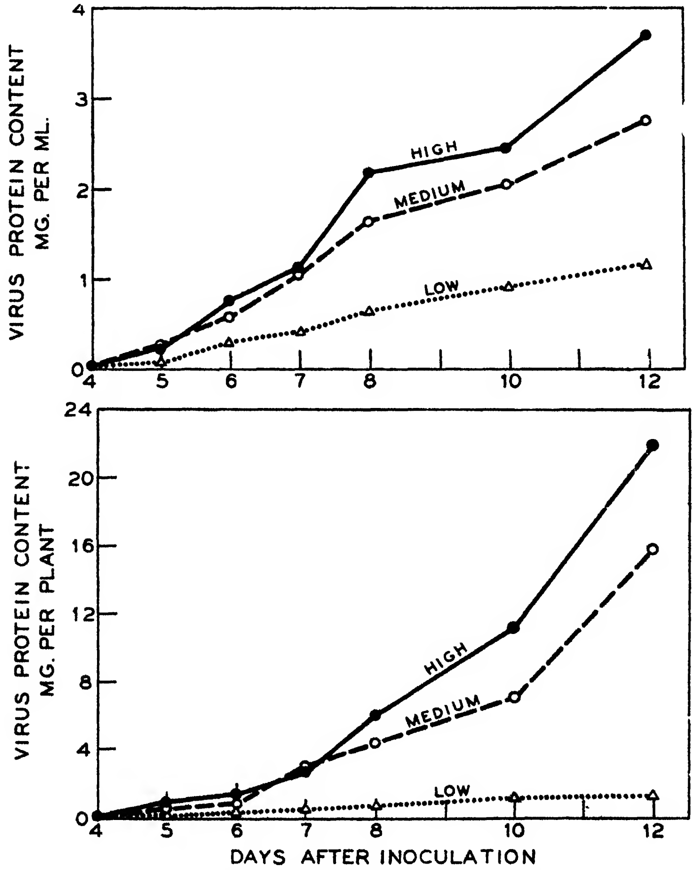

FIG. 2. Virus-protein content of juice from plants harvested at 4-, 5-, 6-, 7-, 8-, 10-, and 12-day intervals after inoculation, the plants having received either low, medium, or high amounts of nitrogen. The upper graph represents the virus-protein contents per ml. of juice; the lower graph the virus-protein contents per plant.

The data pertaining to the virus-protein content in the juice from plants receiving either low, medium, or high amounts of nitrogen are represented by the 2 graphs in figure 2. As shown in the upper graph, the juice from the low-nitrogen plants at all harvests had less virus protein per ml. than that from plants on either of the other 2 nitrogen treatments; juice from the high-nitrogen plants had the most. Four days after inoculation, virus-protein content per ml. of expressed juice was practically the same in all 3 treatments. During the next 8 days, the virus-protein content per ml. of juice from the low-nitrogen plants increased 12-fold, whereas that in the medium-nitrogen and that in the high-nitrogen plants increased over 27 and 36 times, respectively. During the 8-day interval between the 4th and 12th days after inoculation, the calculated virus-protein content in juice from the entire low-nitrogen plants increased over 20 times. During this same interval, however, the virus protein in the medium-nitrogen plants increased over 130 times, and that in the high-nitrogen plants over 200 times. The growth data in table II show that in no case did the weight of these plants increase more than 5-fold during this interval.

Response in older plants

Since the above data show that in young plants an increase in the nitrogen supply is quickly followed by a rapid increase in virus content, an experiment was next carried out to determine what effect a supplementary supply of nitrogen might have when applied to older plants. High-nitrogen treatment was started on groups of representative plants at 4 different intervals after inoculation. The relative size of the plants in each group when the supplementary nitrogen was first added is illustrated in figure 3.

Fig. 3. Representative tobacco plants at time of initiation of high-nitrogen application, 0, 6, 12, and 18 days after inoculation, respectively, from left to right. (Photograph by J. A. Carlile.)

In series A, represented by the plant on the left, the high-nitrogen solution was applied every second day beginning on the day the plants were inoculated. In series B, the first addition of the high-nitrogen solution was made 6 days after inoculation; in series C, 12 days after; and in series D, 18 days after inoculation. Since the experimental data obtained in series D were similar to those in series C, they will not be included in the present discussion except for occasional mention. Plants not receiving the high-nitrogen solution were supplied the medium-nitrogen solution every second day. All plants were frozen immediately after harvesting so that determinations could be made of the virus-protein content. The results of this experiment, carried out during September and October, are recorded in table III.

TABLE III

INCREASE IN VIRUS CONCENTRATION AND VIRUS-PROTEIN CONTENT OF JUICE OF DISEASED PLANTS FOLLOWING THE ADDITION OF AN INCREASED NITROGEN SUPPLY AT INTERVALS AFTER INOCULATION

INITIAL HIGH-NITROGEN ADDITION: DAYS AFTER INOCULATION	TIME OF HARVEST: DAYS AFTER CHANGE IN N TREATMENT	GREEN WEIGHT PER PLANT		PERCENTAGE INCREASE IN VIRUS CONTENT IN HIGH-N PLANTS	VIRUS-PROTEIN CONTENT PER ML. OF JUICE (MG. N × 6)		
		MEDIUM-N	HIGH-N		MEDIUM-N	HIGH-N	PERCENTAGE INCREASE
days	*days*	*gm.*	*gm.*	%	*mg.*	*mg.*	%
Series A							
0	4	0.8	0.8	23	0.14?	0.03?	
0	8	2.0	1.7	48	1.08	1.39	28
0	12	4.5	3.6	39	1.84	2.52	37
Series B							
6	4	3.0	3.5	36	1.74	1.91	10
6	8	5.2	5.5	71	2.18	3.19	46
6	12	9.5	10.0	97	2.60	3.83	47
Series C							
12	4	7.8	6.9	0	3.10?	3.33	8
12	8	16.2	14.1	7	2.75	3.70	34
12	12	23.2	17.8	55	2.86	3.88	36

The plants in series A were quite small, since the initial addition of the high-nitrogen solution was made at the time of inoculation. At 4-day intervals after inoculation, representative plants were harvested from both the high- and medium-nitrogen treatments. At the first harvest, the juice from the high-nitrogen plants was 23 per cent. more active than that from the medium-nitrogen plants. After 8 days it was 48 per cent. more active and 4 days later 39 per cent. more active. In series B, the initial addition of the high-nitrogen solution was not made until the 6th day after inoculation, at which time the plants showed symptoms of systemic infection. With plants at this stage, the high-nitrogen treatment brought about a pronounced increase in the virus activity of the juice. Four days after the

change in nutrient solution, juice from the high-nitrogen plants was 36 per cent. more active than that from the medium-nitrogen plants. It was 71 per cent. more active after 8 days and 97 per cent. more active after 12 days. This percentage increase is greater than that recorded in series A. This may be explained by the fact that in series A the plants were inoculated at the same time that high-nitrogen treatment was started. Therefore, several days were required for the virus to become established and to invade the plants systemically before it could respond to the added supply of available nitrogen. In series B, however, the plants were thoroughly diseased before the addition of the supplementary supply of nitrogen. In the third group (series C) the plants had been inoculated for 12 days before they received the high-nitrogen solution. With these larger plants, the high-nitrogen treatment apparently produced little or no increase in virus activity until the plants had been on treatment about 12 days. It may be that in larger plants the increased nitrogen supply does not become effective as rapidly as it does in medium-size plants. A similar relationship was found when the high-nitrogen treatment was started on the 18th day after inoculation.

The virus-protein content of the juice from plants 4 days after inoculation, before symptoms of systemic invasion had become apparent, was practically negligible. Eight days after inoculation, when the plants showed symptoms of systemic invasion, the virus-protein concentration in the juice of the high-nitrogen plants was 28 per cent. higher than that in the medium-nitrogen control plants. Four days later it was 37 per cent. higher in the high-nitrogen plants. This percentage increase in virus protein is comparable to the increase found in the virus activity studies.

In series B, with systemically diseased plants, the juice from the high-nitrogen plants had a higher virus-protein content than did the medium-nitrogen plants at each of the 3 harvests. The percentage increase became greater as the period of high-nitrogen treatment became more extended. Although the percentage increase in virus protein, due to the high-nitrogen treatment, was smaller than the percentage increase recorded by the activity measurements, both were in the same direction. With older plants (series C) the high-nitrogen treatment also resulted in the formation of a higher virus-protein concentration in the juice of diseased plants. The nitrogen measurements of the medium-nitrogen treatment 4 days after treatment were abnormally high and may not be representative, but those made after 8 and 12 days indicate that the virus-protein concentration in the juice from the high-nitrogen plants was 35 per cent. greater than that in juice from the medium-nitrogen plants.

Discussion

The experimental data presented in this paper are at variance with

recent results published by RISCHKOV and SMIRNOVA (7). Working with tomato plants infected with tobacco-mosaic virus, they reported that (1) the concentration of virus in the juice of nitrogen-deficient plants is equal to that in plants supplied normal amounts, and that (2) the disease fails to retard the development of plants receiving an adequate nitrogen supply. It is difficult to reconcile the results of these workers with those obtained by the writer. The data presented here (table I) and in a previous paper (**10**, table III) indicate a much lower virus concentration in nitrogen-deficient plants, the concentration often amounting to as little as 1/30 that in nitrogen-fed plants. Previously published data (**10**, table V), corroborated by similar unreported data found in this study, also show that, on all nitrogen levels tested, diseased plants are regularly smaller and weigh less than healthy control plants.

Throughout this paper, the experimental data dealing with rate of virus multiplication have been based on measurements of the relative virus content in the juice of treated and control plants at definite periods following inoculation. Since these time periods were short, it is assumed that any variation in relative virus content was due primarily to an alteration in the rate of virus multiplication brought about by the change in nutritional treatment. Although the experimental evidence supports this hypothesis, two other possible explanations should be mentioned briefly. One possibility is that the increased virus content of the high-nitrogen plants may have resulted from a greater stability of virus in these plants than in low-nitrogen plants, implying a differential rate of inactivation rather than a differential rate of formation. KUNKEL found that such viruses as those causing aster yellows (**5**), peach yellows, little peach, peach rosette, and red suture (**4**) can be inactivated by heat treatments within the living plant. ROSS (**8**) suggested that alfalfa-mosaic virus may be inactivated in tobacco plants under normal growing conditions. It has recently been shown (**11**) that the activity of tobacco-mosaic virus *in vivo* may decrease under nitrogen-deficient conditions, but such a decrease could account for only a small part of the differences observed here between the virus activity of the plants on the various nitrogen treatments.

Another possibility that might explain the experimental evidence is that under certain growing conditions the virus particles within the plant or in the expressed juice may come together to form aggregates containing many virus particles. If this assumption were correct, the high-nitrogen treatment might cause dispersal of virus particles into smaller aggregates, thereby producing more infectious units without actually increasing the total amount of virus present. The fact that in this study a corresponding increase was found not only in virus concentration as determined by the local-lesion method but also in virus-protein content would tend to rule out differential dispersion as an explanation for the observed facts.

Therefore, in the present state of our knowledge, it is permissible to state with certainty only that the high-nitrogen treatment has resulted in an increase in the virus content of the expressed juice. In the light of present evidence, it would seem logical to assume that an increase in the rate of virus multiplication may have taken place in the high-nitrogen plants. The increase appears to be correlated with the nitrogen supply itself and not in any direct way with a growth differential resulting from the nitrogen treatment. It is not improbable that even in small plants there may be only a limited supply of available nitrogen. In this case, as with larger plants, the competition for the available nitrogen between the normal growth processes and those responsible for virus formation may be a limiting factor in virus multiplication. With an abundant supply of nitrogen and in the absence of other possible limiting factors, virus multiplication should proceed at an ever increasing rate, the nitrogen serving as part of the substrate.

Summary

A study was made of the influence of nitrogen supply on the rate of multiplication of tobacco-mosaic virus in Turkish tobacco plants. Plants, grown in sand, were supplied with nutrient solutions containing either a low, medium, or high amount of nitrogen. Assay for virus activity was made on *Phaseolus vulgaris* L. var. Early Golden Cluster. The virus protein was isolated by means of an air-driven ultracentrifuge.

In young plants a difference in virus activity could be detected as early as the 5th day after inoculation, when the juice expressed from low-nitrogen plants was found to be only about 35 per cent. as active as that in juice from plants receiving more nitrogen. By the 8th day, it was less than 25 per cent. as active. A 3-fold difference was recorded in the virus-protein contents in the juices of these 2 treatments. The activity data calculated per plant indicate that by the 8th day after inoculation the juice from the high-nitrogen plants contained 12 times more virus than that from the low-nitrogen plants, even though the high-nitrogen plants were only 2 or 3 times as large. From the 4th to the 12th day after inoculation, the virus-protein content of the juice expressed from the low-nitrogen plants increased about 20 times, whereas that of juice from the high-nitrogen plants increased over 200 times.

Older diseased plants receiving the medium-nitrogen solution for longer periods before being given the high-nitrogen solution attained greater virus activity as a result of the supplementary supply of nitrogen. The data indicate that the larger the plant the more time is required for the increased nitrogen supply to become effective.

The experimental evidence is interpreted as supporting the view that the increased virus activity associated with an increased nitrogen supply

is due primarily to an increase in the rate of virus multiplication in the case of the high-nitrogen plants and only slightly, if at all, to the partial inactivation of the virus entity in the case of the low-nitrogen plants.

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THE EFFECT OF HIGH CONCENTRATIONS OF CARBON DIOXIDE ON THE GLUTAMINE AND ASPARAGINE METABOLISM OF THE SUGAR BEET

J. M. Fife and W. C. Ferguson

(WITH ONE FIGURE)

Introduction

It is well known that asparagine is one of the most important amides of the cell sap of plants and that excesses of ammonia from any source (internal or external) are removed in part by conversion into asparagine (**1**, **8**). Likewise, glutamine is an important amide and in some plants its formation may be more important in maintaining ammonia at a tolerant level than is asparagine (**8**, **12**, **13**).

Earlier investigations (**3**) revealed that in sugar beet plants subjected to high concentrations of carbon dioxide there is an increase in ammonia and a decrease in the hydrogen ion concentration of the cell sap (**3**, **4**). The increase of ammonia, in the plants exposed to 20 per cent. carbon dioxide, was fully accounted for by the loss of amide nitrogen. Only 44 per cent. of the increase of ammonia nitrogen could be accounted for by the loss of amide nitrogen when the plants were exposed to 40 per cent. carbon dioxide. Only 10 per cent. of the increase of ammonia could be accounted for by the loss of amide nitrogen, when 80 per cent. carbon dioxide was used. It was also demonstrated that when carbon dioxide-treated beet plants were allowed to recover, the amide nitrogen increased to its original value apparently at the expense of the ammonia and with the formation of hydrogen ions. Inasmuch as most plants are poorly buffered at the pH of their normal cell sap, it may be that amide nitrogen plays an important rôle in the detoxification of hydrogen ions or compounds formed as a result of the treatment of plants with carbon dioxide.

In this paper are presented data showing the changes in ammonia, glutamine-amide and asparagine-amide nitrogen in sugar beet plants exposed to high concentrations of carbon dioxide. Data are also presented showing the changes which take place in beet-leaf extracts[1] on standing and certain modifications of the method of treating the extracts which appear to be a distinct advantage in determining more accurately the mentioned compounds in beet leaf extracts.

The methods developed by Vickery, Pucher, Clark, Chibnall and Westall (**11**) for the determination of glutamine in the presence of asparagine in plant tissues were used, with a slight modification, in studying

[1] The term "beet-leaf extracts" refers to extracts of the blades of the leaves only.

the rôle these compounds play in the reactions accelerated in plants by high concentrations of carbon dioxide. They were further used to determine, if possible, the source of the ammonia not accounted for by the decrease in amide nitrogen reported in earlier work (3). Preliminary tests revealed that modification was necessary; in the nitrogen fractions of beet-leaf extracts prepared by those methods (VICKERY *et al.*), changes occurred before complete analysis could be made of all the extracts comprising one experiment.

Materials and methods

Sugar beet plants were grown in the greenhouse in six-inch pots (4 plants per pot) in well fertilized soil for 6 to 8 weeks. The tests in which unfertilized plants were used received no ammonium sulphate. The heavily fertilized plants received ammonium sulphate each week to keep the plants well supplied with nitrogen. Each pot also received a heavy application of ammonium sulphate 10 to 14 days before the samples were taken in order to stimulate the production of amides (12). A large number of plants were pulled and divided into lots. No tests were conducted in which fewer than 30, and generally 50, plants made up the samples. The sampling error was found to be insignificant when 20 plants comprised the sample. One lot served as the control while the remaining lots were treated with different concentrations of carbon dioxide and for varying lengths of time.

The plants were placed in an upright position in a test tube basket over which was placed a 22-liter bell jar. Air equal to the desired volume of carbon dioxide was displaced with water. Carbon dioxide from a cylinder was immediately forced into the bell jar until all the water was displaced. The entire plants were treated in this way for a definite period; the blades and petioles were then immediately removed, separated, and ground in a plate type grinding mill. Certain lots were allowed to recover from the carbon dioxide treatment; these were placed in the laboratory with their roots in water for 5 hours, after which the leaves were removed. From this point on, the methods of preparing the samples and also the analytical procedure were identical for the treated and the control samples; consequently all systematic errors were eliminated by mutual cancellation.

The pulp was thoroughly mixed before 100-gram samples and two moisture samples were removed. Twenty-five ml. of ether were added to the samples which were allowed to stand, with frequent stirring, for 30 minutes. Each sample was then transferred to a coarse-meshed cloth, and the juice expressed by hand. Water was then added to the pulp and the liquid expressed by hand. This operation was repeated six times to insure the removal of all soluble nitrogen. This brought the total volume of the extracts to approximately 250 ml. Tests showed that an average of 80 per cent. of the liquid present in the pulp was expressed after each addition of

water. A further test showed that only 0.34 per cent. of the total soluble nitrogen present in the sample was found in the 7th, 8th and 9th extractions combined. The samples were brought to 80° C., filtered through paper pulp, cooled rapidly, and made up to a volume of 250 ml. Special tests showed that the paper pulp did not retain significant amounts of soluble nitrogen.

All nitrogen determinations were made in duplicate. The ammonia was distilled over at atmospheric pressure in a micro Pyrex steam distillation apparatus (5). The distillates were evaporated to approximately 20 ml. and titrated using 0.01 N solutions and methyl red as indicator. Correc-

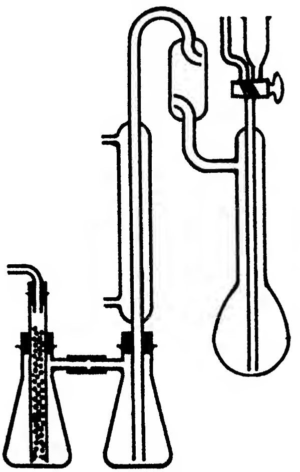

FIG. 1. Vacuum distillation apparatus.

tions for the blank were applied in all determinations. The titration and distillation error using this apparatus was 0.014 mgm. of nitrogen.

The ammonia in 100-ml. aliquots was determined by distilling *in vacuo* unless otherwise stated. Preliminary experiments demonstrated that a bead tower as shown in figure 1 was necessary to hold the ammonia carried over with the spray caused by the air bubbling through the liquid in the receiving flask. The sample was transferred to the distilling apparatus through the stopcock and 25 ml. of a 0.5 N sodium hydroxide solution containing 5 per cent. borax was added, with distilled water for dilution, to render the sample alkaline. Pressure was reduced in the apparatus by connecting the outlet of the bead tower to an aspirator. A small amount of heat was applied and the stopcock opened allowing sufficient air to bubble through the sample to maintain the pressure at 70 mm. of mercury. A small amount of acid was added to the receiving flask and the flask holding the bead tower. The distillate and the acid in the bead tower were combined and redistilled in the micro Pyrex steam distillation apparatus.

This was done to eliminate any alkali which might have been carried over in the spray formed by the air bubbling through the sample.

Glutamine-amide nitrogen was determined by the method described by VICKERY *et al.* (**11**). To a 25-ml. aliquot was added an equal volume of a 0.2 M phosphate buffer mixture (prepared according to CLARK [(**2**) p. 210, pH 6.468] and hydrolyzed for two hours. The distillation was carried out *in vacuo* as described, using the alkaline borate mixture to render the sample alkaline. The distillates were redistilled in the micro steam distillation apparatus. Preliminary tests as suggested by VICKERY *et al.* (**11**) indicated the absence of urea and allantoin in the beet-leaf extracts.

Samples for asparagine-amide nitrogen were made 1 normal with sulphuric acid and hydrolyzed for three hours. The samples were almost neutralized with sodium hydroxide and then made slightly alkaline with the alkaline borate mixture; ammonia was distilled over at atmospheric pressure. Extensive tests demonstrated that with the material and the apparatus used it was not necessary to distill the asparagine-amide samples over *in vacuo.*

Soluble nitrogen was determined on aliquots after precipitation of the proteins. A catalyst of sodium sulphate, copper sulphate, and selenium was used to hasten digestion (**6**).

Results

Preliminary experiments indicated that certain nitrogen fractions in beet-leaf extracts were highly unstable upon standing several hours under laboratory conditions, as shown by an increase in ammonia nitrogen. Preliminary tests also revealed that heating the extracts to 80° C. and filtering, as outlined by VICKERY *et al.* (**11**) was insufficient to prevent these changes from taking place in the extracts before complete analysis could be made of all the extracts comprising one experiment. The rate and extent to which these changes take place in aliquots of beet-leaf extracts on standing are shown in table I. These data are typical of the results obtained. In 24 hours, the ammonia increased 3.8 per cent., while the glutamine-amide nitrogen decreased the same amount. In the 72-hour period the ammonia increased 135 per cent. Approximately 20 per cent. of the total gain in ammonia nitrogen could be accounted for in this experiment by the loss in glutamine-amide nitrogen. During this same period the asparagine-amide nitrogen increased 40 per cent.

In all cases a marked increase in ammonia occurred upon standing. The glutamine-amide nitrogen consistently decreased while at the same time the asparagine-amide nitrogen increased. The increase in asparagine-amide nitrogen, on standing 48 hours, for the experiments as listed was 39, 97, 114 and 22 per cent., respectively. In view of these facts, it is evident that cer-

TABLE I

THE CHANGES IN AMMONIA, GLUTAMINE-AMIDE AND ASPARAGINE-AMIDE NITROGEN IN ALIQUOTS OF BEET-LEAF EXTRACTS HEATED AND ALLOWED TO STAND

Hours before analysis	Temperature heated	Nitrogen per 1000 grams of dry tissue				
		Soluble nitrogen	NH_3 nitrogen	Glutamine-amide nitrogen	Asparagine-amide nitrogen	Total amide nitrogen
	°C.	gm.	gm.	gm.	gm.	gm.
Control	80	13.58	0.368	0.944	0.500	1.444
24	80		0.382	0.908	0.449	1.357
48	80		0.526	0.905	0.695	1.600
72	80	13.58	0.865	0.847	0.701	1.548
Control	80	10.20	0.417	0.530	0.288	0.818
48	80	10.20	1.007	0.359	0.566	0.925
Control	80	11.92	0.382	0.776	0.494	1.270
48	80	11.92	0.832	0.146	1.057	1.203
Control	100	10.91	0.265	0.371	0.388	0.759
48	100	11.40	0.642	0.272	0.474	0.746

tain substances which are unstable are still present in the beet-leaf extracts after being heated to 80° C. and filtered. WEBSTER (**14**) observed the formation of ammonia in plant extracts stored in alcohol and concluded that it might have come from deaminization of amino acids.

Inasmuch as the pH of the beet-leaf extracts was higher than the isoelectric points of asparagine and glutamine, the next step was to determine the nitrogen changes in the extracts on standing after the hydrogen ion concentration had been increased. In two experiments the extracts used as controls were heated to 80° C. and filtered. The other extracts were brought to boiling and 2 ml. of 10 per cent. acetic acid added as recommended by

TABLE II

THE CHANGES IN AMMONIA, GLUTAMINE AMIDE, AND ASPARAGINE-AMIDE NITROGEN, IN THE BEET LEAF EXTRACTS WHEN HEATED TO 80° C. AND 100° C. AND ADJUSTED TO PH 4.5

Temperature		pH	Stood before analysis	Nitrogen per 1000 grams dry tissue				
				Soluble	NH_3	Glutamine amide	Asparagine amide	Total amide
°C.			hr.	gm.	gm.	gm.	gm.	gm.
80	Control	5.9	0	14.68	0.412	0.900	0.638	1.538
100		4.5	0	14.42	0.428	0.940	0.654	1.594
80	Control	5.9	0	14.72	0.924	1.176	0.469	1.645
100		4.5	0	13.98	0.920	1.152	0.465	1.617
80	Control	5.9	0	10.91	0.265	0.371	0.388	0.759
80		4.5	48	11.12	0.275	0.382	0.421	0.803
100		5.9	48	11.40	0.642	0.272	0.474	0.746

NIGHTINGALE *et al.* (7). The samples were then filtered, cooled, and analyzed immediately. In a third experiment, the control extract was heated to 80° C., filtered, and analyzed immediately. Another extract was heated to 80° C., 2 ml. of 10 per cent. acetic acid added, filtered, and allowed to stand 48 hours before being analyzed. A third extract was heated to 100° C., filtered, and allowed to stand 48 hours before analysis was made. The data are shown in table II.

The first two experiments demonstrate that the elevated temperature with the higher hydrogen ion concentration has no effect on the nitrogen fractions. The slight differences shown are probably due to variations in extraction. The third experiment demonstrates that the breakdown of glutamine and other amides, with the formation of ammonia, is not arrested by merely bringing the extract to boiling. If the hydrogen ion concentration was increased no significant change in the nitrogen fractions occurred in the extract when allowed to stand 48 hours.

It appears that the catalytic agents responsible for the breakdown of glutamine and the formation of asparagine-amide nitrogen are not inactivated by bringing the extracts to boiling. If the hydrogen ion concentration of the extract is increased sufficiently to bring the pH to 4.5 (which is below the isoelectric point of glutamine and asparagine) the extract is apparently rendered stable.

TABLE III

THE EFFECT OF 20 AND 40 PER CENT. CARBON DIOXIDE ON CERTAIN NITROGEN FRACTIONS IN THE BLADES OF SUGAR BEET LEAVES

Treatment			Nitrogen per 1000 grams of dry tissue				
Carbon dioxide		Period	Soluble	Ammonia	Glutamine amide	Asparagine amide	Total amide
%		*Min.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
	Control		13.66	0.248	0.651	0.609	1.260
20		90	13.18	0.410	0.518	0.475	0.993
	Recovered		12.55	0.330	0.531	0.591	1.122
	Control		11.90	0.382	0.776	0.494	1.270
20		90	12.30	0.523	0.719	0.455	1.174
	Recovered		12.60	0.487	0.908	0.503	1.411
	Control		9.28	0.141	0.422	0.298	0.720
40		60	9.62	0.268	0.384	0.284	0.668
	Control		10.91	0.265	0.371	0.388	0.759
40		60	9.90	0.400	0.222	0.319	0.541
	Recovered		11.12	0.257	0.435	0.388	0.823
	Control		9.18	0.137	0.239	0.235	0.474
40		60	9.06	0.242	0.225	0.200	0.425
	Recovered		9.07	0.151	0.215	0.236	0.451

After the preliminary ground work was settled, a number of experiments were conducted in which sugar beet plants were treated with carbon dioxide in concentrations of 20, 40, 80, and 100 per cent.

The experiments conducted at 20 and 40 per cent. carbon dioxide are shown in table III. Changes in the nitrogen fractions in general are the same. In all experiments conducted at these concentrations the ammonia nitrogen increased while the glutamine-amide and asparagine-amide nitrogen decreased. In general, the loss in amide nitrogen could be accounted for by the gain in ammonia nitrogen. When the treated plants were exposed to a normal atmosphere, the reactions appeared to be reversed since the nitrogen fractions in all experiments approached their original values. No significant change was observed in the soluble nitrogen when the plants were subjected to these concentrations of carbon dioxide.

Changes in the nitrogen fractions in the plant in 80 and 100 per cent. carbon dioxide are different in certain respects from the reactions in 20 and 40 per cent. carbon dioxide (table IV). In general, the increase in am-

TABLE IV

THE EFFECT OF 80 AND 100 PER CENT. CARBON DIOXIDE ON CERTAIN NITROGEN FRACTIONS IN THE BLADES OF SUGAR BEET LEAVES

Treatment		Nitrogen per 1000 grams of dry tissue				
Carbon dioxide	Period	Soluble	Ammonia	Glutamine amide	Asparagine amide	Total amide
%	*min.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
	Control	12.18	0.871	0.841	0.349	1.190
80	90	14.49	1.853	0.964	0.414	1.378
	Recovered	11.22	0.627	0.778	0.313	1.091
	Control	13.47	1.407	1.041	0.428	1.469
80	90	12.45	1.041	0.900	0.458	1.358
	Recovered	12.92	0.708	1.150	0.442	1.592
	Control	13.27	0.575	0.824	0.459	1.283
80	90	12.72	0.716	0.678	0.475	1.153
	Control	13.42	0.975	0.817	0.655	1.472
100	90	14.25	0.819	0.694	0.697	1.391
	Recovered	12.33	0.590	0.607	0.527	1.134
	Control	11.45	0.379	0.460	0.393	0.853
100	90	12.10	0.646	0.283	0.653	0.936
	Recovered	12.07	0.839	0.497	0.424	0.921
	Control	13.97	0.789	1.435	0.484	1.919
100	90	14.88	0.936	1.116	0.518	1.634
	Recovered	14.03	0.612	1.291	0.429	1.720
	Control	10.47	0.253	0.420	0.295	0.715
100	90	11.59	0.486	0.371	0.306	0.677
	Recovered	10.75	0.218	0.370	0.354	0.724

monia nitrogen was much greater at 80 and 100 per cent. than at 20 and 40 per cent. carbon dioxide. Also, the loss in glutamine-amide nitrogen was much greater at 80 and 100 per cent. carbon dioxide than at the lower concentrations. This decrease in glutamine-amide nitrogen does not account in full for the observed gain in ammonia. A striking point is the difference between the response of the plants to the higher and the lower concentrations of carbon dioxide in regard to the asparagine-amide nitrogen. At the higher concentrations a significant increase in asparagine-amide nitrogen occurred in all experiments—66 per cent. in one instance. In contrast to this, there was a decrease in asparagine-amide nitrogen in all experiments with the lower concentrations and as much as a 22 per cent. decrease in one case. In general, there was an increase in soluble nitrogen at 80 and 100 per cent. carbon dioxide. When the plants, exposed to these concentrations of carbon dioxide, were placed in a normal atmosphere for 5 hours the nitrogen fractions did not revert to the values obtained with the untreated plants.

In certain of the earlier experiments, blades and petioles were analyzed together because the plant material available was limited. When these data (table V) are compared with those obtained in tables III and IV, in

TABLE V

THE EFFECT OF 20 PER CENT. CARBON DIOXIDE FOR TWO HOURS ON CERTAIN NITROGEN FRACTIONS IN THE BLADES AND PETIOLES OF SUGAR BEET LEAVES[a]

	Nitrogen per 1000 grams of dry tissue				
	Soluble	Ammonia	Glutamine amide	Asparagine amide	Total amide
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
Control	....	0.726	0.751	0.311	1.062
Treated	....	0.995	1.506	0.401	1.907
Control	8.80	0.197	1.050	0.294	1.344
Treated	9.70	0.302	1.472	0.368	1.840
Control	8.51	0.133	0.718	0.073	0.791
Treated	9.10	0.212	0.978	0.120	1.090
Control	10.10	0.194	0.522	0.380	0.902
Treated	10.90	0.334	0.580	0.421	1.001
Control	6.41	0.270	0.393	0.213	0.606
Treated	6.80	0.395	0.512	0.247	0.759

[a] The plants used in these experiments were grown in greenhouse soil without the applications of ammonium sulphate.

which only the blades made up the sample, it is strongly indicated that the glutamine and asparagine-amide nitrogen changes may be greatly influenced by the composition of the different parts of the leaf. For example,

when the blades only made up the sample (table III) there was a decrease in glutamine and asparagine-amide nitrogen in all experiments. When the sample contained both blades and petioles (as in table V) striking increases in glutamine and asparagine-amide nitrogen were obtained in every case. Similar increases in asparagine-amide nitrogen were noted (when blades only comprised the sample) only at 80 and 100 per cent. carbon dioxide (table IV).

In view of this evidence, experiments were conducted to determine the relative amounts of glutamine and asparagine present in the blades and petioles, and at the same time to observe the changes in these nitrogen fractions occurring simultaneously in the two parts of the leaf under the influ-

TABLE VI

THE EFFECT OF 20 PER CENT. CARBON DIOXIDE FOR TWO HOURS ON CERTAIN NITROGEN FRACTIONS IN THE BLADES AND PETIOLES, ANALYZED SEPARATELY AND COMBINED*

	Nitrogen per 1000 grams of dry tissue				
	Soluble	Ammonia	Glutamine amide	Asparagine amide	Total amide
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
Blades, Control	13.98	0.537	0.952	0.591	1.543
Petioles, Control	13.00	1.212	3.868	0.170	4.038
Blades, Treated	14.54	0.759	0.777	0.566	1.343
Petioles, Treated	15.22	1.395	3.787	0.638	4.425
Blades and Petioles, Control†	13.52	0.858	2.337	0.398	2.735
Blades and Petioles, Treated†	14.97	1.059	2.260	0.600	2.860
Blades, Control	11.32	0.376	0.508	0.458	0.966
Petioles, Control	9.83	0.800	2.915	0.535	3.450
Blades, Treated	10.87	0.432	0.459	0.391	0.850
Petioles, Treated	9.58	0.956	2.619	0.599	3.218
Blades and Petioles, Control‡	10.27	0.609	1.453	0.466	1.919
Blades and Petioles, Treated‡	10.26	0.730	1.332	0.554	1.886
Blades, Control	9.74	0.352	0.526	0.558	1.084
Petioles, Control	10.24	0.767	2.753	0.565	3.318
Blades, Treated	10.95	0.500	0.484		
Petioles, Treated	9.94	1.062	2.391		
Blades and Petioles, Control§	10.97	0.487	1.710	0.633	2.343
Blades and Petioles, Treated§	10.27	0.672	1.402		
Blades and Petioles, Control§	8.30	0.359	1.549	0.442	1.991
Blades and Petioles, Treated§	8.34	0.759	1.158	0.478	1.636
Blades and Petioles, Control§	10.39	1.376	2.482	0.518	3.000
Blades and Petioles, Treated§	11.63	1.918	1.640	0.634	2.274

* Plants heavily fertilized.

† Calculated and corrected to the exact dry weight ratio from the values for the above.

‡ Determined from a mixture of equal amounts of blades and petioles; values then corrected to the exact dry weight ratio of blades to petioles.

§ Determined from 100 grams of ground blades and petioles.

ence of 20 per cent. carbon dioxide. The blades and petioles were separated after a 2-hour treatment and analyzed separately. The results obtained are shown in table VI. From these data it is evident that the glutamine and the asparagine content in the blades and petioles is vastly different. Calculations also show that 80, 85, and 84 per cent., respectively, of the total glutamine-amide nitrogen in the blades and petioles are found in the petioles.

The nitrogen changes at 20 per cent. carbon dioxide in the blades, in these experiments, also confirm those shown in table III.

The difference between the results given in table V and those in table VI corresponds to the difference in nutritional levels between the two lots of plants involved. When whole leaves of the unfertilized beets were treated with 20 per cent. carbon dioxide and then analyzed, a striking increase in glutamine-amide nitrogen over the amount in the untreated leaves was found (table V). Conversely, when whole leaves of the heavily fertilized beets were similarly treated and analyzed a decrease in the glutamine-amide nitrogen from that in the controls was noted (table VI). Further study of the two tables reveals that in the unfertilized plants the untreated leaves contained only about one-third as much glutamine-amide nitrogen as did the untreated leaves of the fertilized plants; also that with leaves low in glutamine-amide nitrogen the amount of it was strikingly increased by the carbon dioxide treatment, whereas with leaves high in this constituent the amount was decreased by the carbon dioxide.

Discussion

The reactions occurring in beet-leaf extracts on standing appear to be similar to those produced in the blades by the higher concentrations of carbon dioxide. There appear to be at least two hydrolytic reactions occurring in beet plant extracts (when allowed to stand) and in the cell sap of beet leaves when the leaves are exposed to high concentrations of carbon dioxide. The first reaction, which may be responsible for the observed increase in ammonia nitrogen, appears to be mainly the hydrolysis of glutamine. There is evidence that asparagine may also be slowly hydrolyzed to yield ammonia in blade extracts when allowed to stand for a period of 24 hours. A consistent decrease in asparagine-amide nitrogen was noted when blades were subjected to 20 and 40 per cent. carbon dioxide. That asparagine is slowly hydrolyzed to yield ammonia is further supported by the fact that the decrease in glutamine-amide nitrogen does not fully account for the gain in ammonia.

The hydrolysis of glutamine and the small amount of ammonia that may come from the slow breakdown of asparagine does not account for all the increase in ammonia nitrogen when the plants are exposed to concentrations of carbon dioxide greater than 40 per cent.

The second hydrolytic reaction apparently involves substances more complex than amides. These substances, probably peptides (or other similar substances) are hydrolyzed to yield nitrogen which is determined as asparagine-amide and glutamine-amide nitrogen. This reaction may yield sufficient ammonia to account for the excess ammonia not coming from the breakdown of glutamine and asparagine. If the substance is a peptide and contains only asparagine, then the ammonia not accounted for by the loss in glutamine would come from the hydrolysis of asparagine. The rate of hydrolysis of asparagine would, however, be much slower than that of the peptide to account for the net increase in asparagine. If, however, the peptide contains both glutamine and asparagine, then the glutamine present must be hydrolyzed to form glutamic acid and ammonia faster than the peptide is broken down to yield glutamine to account for the net decrease in glutamine-amide nitrogen. This would account for all the ammonia and the increase in asparagine-amide nitrogen.

The distribution and amount of certain nitrogen fractions in normal blades and petioles may help to explain certain changes effected in the beets when treated with carbon dioxide. The asparagine-amide nitrogen appears to be nearly evenly distributed between the blades and petioles. On the other hand the glutamine-amide nitrogen and other nitrogen constituents (probably peptides) which yield glutamine-amide nitrogen on carbon dioxide treatment are more abundant in the petiole. In the case where an increase in glutamine-amide nitrogen was observed (unfertilized blades and petioles treated with 20 per cent. carbon dioxide, table V), the breakdown of the peptide to form glutamine was faster than the hydrolysis of glutamine-amide nitrogen to ammonia.

It appears that the chemical changes occurring in plants treated with carbon dioxide may be influenced by the nitrogen nutritional levels at which the plants were maintained. In all cases where the plants were heavily fertilized, a decrease in glutamine-amide nitrogen was noted on carbon dioxide treatment. However, in those experiments (table V) in which the plants had not been fertilized, an increase in glutamine-amide nitrogen was obtained. The experiments indicate that under conditions of low nitrogen fertilization the glutamine may be stored in the plants as soluble peptides and that upon carbon dioxide treatment these peptides may be rapidly hydrolyzed, which would result in the observed increase in glutamine-amide nitrogen.

There is evidence (**3**) to show that even 100 per cent. carbon dioxide for such short periods as 90 minutes apparently did not seriously affect the normal respiratory processes of the plants. Those tests showed that the expressed cell sap of plants exposed to this concentration of carbon dioxide for one hour increased in pH as did the sap of plants exposed to only 20

per cent. However, when they were exposed for 4 or 5 hours to 100 per cent. carbon dioxide the pH of the cell sap decreased. This indicates that with long periods in an atmosphere of carbon dioxide the oxygen tension within cells may be lowered to a point where anaerobic processes are initiated. SMITH (9) studied the absorption of carbon dioxide of unilluminated leaves and found that the absorption by living leaves was very rapid, ten minutes being sufficient for complete saturation. Practically all of the carbon dioxide was absorbed by the water in the leaves. The water in the leaves was found to absorb carbon dioxide in proportion to the amount of water present and in proportion to the partial pressure of the carbon dioxide. It is generally known that the intensity of respiration in many plants is not markedly changed when the oxygen supply is reduced to one-half that in air. In fact, STICH (10) claims that the absorption of oxygen becomes insufficient for most plants when the concentration in air falls below 5 to 8 per cent. From these considerations it would appear that oxygen concentration in the intercellular spaces would not be so seriously affected that normal respiration could not proceed during the 90 minutes the plants were exposed to an atmosphere of carbon dioxide.

VICKERY *et al.* (11) compared the composition of fresh extracts of leaves and stems of tomatoes to tissue dried at temperatures ranging from 70° to 90° C. These investigators pointed out that the agreement between the values (table VI, p. 2717) obtained for the leaf tissue was satisfactory, and somewhat less satisfactory for the stem tissue. It appears from their data that even during the short drying period there was a tendency for an increase in ammonia and asparagine-amide nitrogen and a slight decrease in glutamine-amide nitrogen. For example, some of their data show that drying the leaf tissue at 80° C. for one hour resulted in an increase of approximately 22 per cent. in ammonia, 14 per cent. in asparagine-amide nitrogen, and a decrease in glutamine-amide nitrogen of approximately 3 per cent. It is quite possible that these differences may be significant, for these changes are in the same direction as that when beet-leaf extracts were allowed to stand for a period of 48 hours at room temperature before analysis as reported in the present paper.

It has long been a question in the nitrogen metabolism of plants as to whether a compound is synthesized in a certain tissue or whether its presence is a result of translocation. The fact that the beet leaf rapidly hydrolyzes the amide group of glutamine and asparagine while under the influence of high concentrations of carbon dioxide and reverses the process when placed in a normal atmosphere indicates that the leaves are capable of synthesizing these compounds. CHIBNALL [(1) p. 205] using an infiltration technique showed that glutamine is formed in the blades of rye grass by the dehydration of ammonium glutaminate. VICKERY (13) has demonstrated that the

same reaction takes place in the roots of the beet plant. It is possible that the formation of glutamine and asparagine from the ammonium ion and the corresponding amino acids is a reaction common to the leaves of many plants. This is supported by the fact that ten other species of plants (3) produced hydroxyl ions in the presence of high concentrations of carbon dioxide, presumably by the hydrolysis of the amide group of glutamine and asparagine.

It is recognized that the general process of ammonia detoxification involves the complex process of respiration and the formation of organic acids from which the amino acids and finally the amides are formed. In view of the rapid rate at which the plant is able to synthesize glutamine and asparagine from ammonia and the corresponding amino acids and reverse the process, it appears that the reactions limiting the rate of ammonia detoxification in the plant may be those involving the synthesis of the organic and the amino acids.

Summary

1. Asparagine-amide nitrogen and ammonia increased in beet-leaf extracts when heated to 80° C., filtered, and allowed to stand, while the glutamine-amide nitrogen decreased.

2. No significant change in the nitrogen fractions occurred when the aliquots were allowed to stand for a period of 48 hours, provided the hydrogen ion concentration was increased from pH 5.9 to 4.5.

3. A large increase in ammonia resulted when the blades of beet leaves were exposed to 20 and 40 per cent. carbon dioxide. This increase in ammonia could be accounted for by the decrease in asparagine-amide and glutamine-amide nitrogen.

4. When the blades were exposed to 80 and 100 per cent. carbon dioxide there was a significant increase in soluble nitrogen, a large increase in ammonia and asparagine-amide nitrogen and a decrease in glutamine-amide nitrogen.

5. When whole leaves were treated with 20 per cent. carbon dioxide and the petioles only were analyzed, there was an increase in ammonia and asparagine-amide nitrogen and a decrease in glutamine-nitrogen.

6. The asparagine-amide nitrogen appeared to be distributed nearly evenly between the petioles and blades, while the glutamine-amide nitrogen was concentrated in the petioles.

7. The degree of nitrogen fertilization of beet plants apparently has an important bearing on their glutamine and asparagine metabolism while under the influence of high concentrations of carbon dioxide. The leaves of heavily fertilized plants responded to a 20 per cent. carbon dioxide treatment by showing a decrease in glutamine-amide nitrogen, while the leaves of unfertilized plants responded with an increase in glutamine-amide nitrogen.

8. Complex soluble substances (probably peptides) were hydrolyzed in the plant, while under the influence of high concentrations of carbon dioxide, yielding asparagine-amide nitrogen and under certain conditions glutamine-amide nitrogen.

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SELECTIVE ABSORPTION OF CATIONS BY HIGHER PLANTS

RUNAR COLLANDER

(WITH THREE FIGURES)

Introduction

For about a hundred years it has been known that plants do not absorb the different constituents of a nutrient solution in the same proportions as they occur in the solution; some salts or ions are taken up in relatively greater amounts than others. The mechanism of this salt selection exerted by the roots is, however, still quite obscure. It seems that one of the first steps towards an understanding of this important process should be a careful study of its results. A close examination of the proportions in which several kinds of ions are absorbed from nutrient solutions of known composition by different plant species should serve as a starting point for attempts to elucidate this process. This information, however, is still lacking. We have known, until now, very little about the proportions in which different ions are absorbed from nutrient solutions, of easily determinable composition, which contain the ions to be investigated in equivalent amounts.

It is the purpose of the present investigation to present such experimental data in suitable form to serve as the basis for a discussion of the nature of the selective activity of the roots of higher plants. To this end plants of a number of species have been cultivated in nutrient solutions containing several cations in equivalent amounts, after which the cation composition of the plants was determined by the method of quantitative spectral analysis. The study was thus limited to cations. Since they are less engaged in the metabolism of the plants the cations seem, in fact, more suitable for such studies than most anions.

A preliminary account of some results of this investigation has already been published (7).

Materials and methods

In order to get a satisfactory idea of the range of variation exhibited by different types of plants in their cation selectivity it was found necessary to extend this investigation to some twenty plant species among the phanerogams representing different ecological types and several taxonomical groups. The following species were studied: *Aster tripolium, Atriplex hortense, A. litorale, Avena sativa, Chenopodium bonus Henricus, Fagopyrum esculentum, Helianthus annuus, Lactuca sativa, Melilotus albus, Nicotiana tabacum, Papaver somniferum, Pisum sativum, Plantago lanceolata, P. maritima, Salicornia herbacea, Salsola kali, Sinapis alba, Solanum lycopersicum, Spinacia oleracea, Vicia sativa,* and *Zea mays.*

The plants were mostly cultivated in large enamel vessels, each containing 50 liters of nutrient solution. The vessels were covered with loosely fitting covers of plywood thoroughly impregnated with paraffin. The covers were perforated with numerous holes in which the seedlings were fixed with cotton-wool. It was thus possible to cultivate about 10 to 20 species in the same vessel under almost exactly identical conditions. The seedlings were obtained by germinating seeds in pure silica sand moistened with tap water of very low salt content. Analyses of the seedlings showed that their salt content, when they were planted in the culture vessel, was negligible as compared with the quantities of salt absorbed during their subsequent growth. The plants were cultivated in a greenhouse, the temperature of which fluctuated between about 18° and 25° C. The air in this greenhouse was pronouncedly dry; the transpiration of the plants must have been considerable. In most cases the seeds were sown at the end of March and the plants harvested about two months later when some of the plants (Pisum) were in bloom. Most of the species, moreover, did not develop flowers. Culture solutions were artificially aerated only in experiments with the solutions IV and V; in these cases the air was forced through filters of sintered glass. A distinct effect of the aeration on the development of the plants could not be detected.

As far as possible the culture solutions contained the cations to be compared in equivalent amounts and also those other ions necessary for the normal development of the plants. The solutions were prepared with glass-distilled water; only for solution I was tap water used. In the following list the salt concentrations are indicated as milligram equivalents per liter of solution.

Solution I: 4 $NaNO_3$ + 4 KH_2PO_4 + 4 $MgSO_4$ + 4 $CaCl_2$ (+ about 0.001 Sr as impurity).

Solution II: 2 $NaNO_3$ + 2 KH_2PO_4 + 2 $RbCl$ + 2 $MgSO_4$ + 2 $Ca(NO_3)_2$ + 0.01 $LiCl$ + 0.01 $MnCl_2$ + an unknown amount of Sr.[1]

Solution III: 4 KH_2PO_4 + 4 $MgSO_4$ + 4 $Ca(NO_3)_2$ + 0.01 $LiCl$ + 0.01 $NaCl$ + 0.01 $SrCl_2$ + 0.01 $MnCl_2$.

Solution IV: 2 KNO_3 + 2 KH_2PO_4 + 0.4 $LiCl$ + 0.4 $NaCl$ + 0.4 $MgSO_4$ + 0.4 $CaCl_2$.

Solution V: 2 $NaNO_3$ + 2 KH_2PO_4 + 0.2 $MgSO_4$ + 0.2 $CaCl_2$ + 0.2 $SrCl_2$ + 0.1 $RbCl$ + 0.1 $CsCl$.

Solution VI: 2 KH_2PO_4 + 2 $Ca(NO_3)_2$ + 2 $MgSO_4$ + 0.05 $NaCl$ + 0.05 $SrCl_2$ + 0.05 $MnCl_2$ + 0.05 $CuSO_4$.

[1] In preparing solution II, 0.0005 milligram equivalents $SrCl_2$ per liter of solution was intentionally added; the Ca salt used probably contained some Sr, thus the final Sr concentration of the solution is not known with certainty. A spectrographic determination of it was regrettably neglected.

To each solution was added some ferro-ammonium sulphate or ferro-tartrate and also a little of the "A–Z" salt solution of HOAGLAND, as quoted by SCHROPP and SCHARRER (20). The solutions were changed only once during the course of each experiment; distilled water was added when necessary and also some iron salt. At the end of most experiments the nutrient solutions were analyzed spectroscopically and also samples of the initial solution. A marked decrease of the concentrations of the cations most strongly absorbed (K, Rb, Mg, Ca, Sr) was sometimes observed. It is doubtful, however, if this factor greatly influenced the results obtained. At any rate, it must have affected all of the plants in a similar fashion, and it would only tend to diminish somewhat the observed difference between the abundantly and scantily absorbed cations.

Solution I was used in order to compare, in a preliminary way, the absorption of Na, K, Mg, and Ca; solution II for comparisons of Na, K, Rb, Mg, and Ca; solution III served to compare, on the one hand, K, Mg, and Ca with each other and, on the other, Na, Sr, and Mn. The amounts of Li taken up from this and also from the other solutions referred to were too small for reliable determination. Solution IV was used for comparisons between Li, Na, Mg, and Ca. The main purpose of solution V was to compare the accumulation of Cs with that of Rb, and of Sr with that of Ca, though the same solution makes possible also comparisons between Na and K, or Mg and Ca. Solution VI served only to compare the absorption of Cu with that of Sr and Mn. Besides the solutions mentioned above, some other culture solutions were used for special purposes.

An investigation of this type is made possible only by the use of quick analytical methods by which the amounts of several cations absorbed by the experimental plants may be determined quantitatively within a reasonable time. In this respect the methods of quantitative spectral analysis are clearly superior to the methods of ordinary chemical analysis. In this investigation only the technique of flame spectrography developed by LUNDEGÅRDH (15) was used. It permitted the determination, without difficulty, of ten different cations: Li, Na, K, Rb, Cs, Mg, Ca, Sr, Mn, and Cu.

When harvested, the plants were first weighed and then dried at about 105° C. and weighed again. They were then ashed with concentrated nitric acid and perhydrol in small Kjeldahl flasks (capacity 50 ml.) of Jena glassware or quartz. (Control experiments showed that the amounts of Na, K, Ca, etc., given off by the glass of the Kjeldahl flasks are practically negligible.) The resulting acid ash solution was diluted with distilled water in a volumetric flask of 10- or 25-ml. capacity after which the insoluble SiO_2 was filtered off. The filtered solutions were stored in small flasks of Jena glassware until used for spectroscopic analysis.

The magnitude of the analytical errors depends on several factors, which

in our determinations were not always constant. The accuracy of the analyses therefore, cannot be generally stated by exact figures. It is, however, possible to get an approximate idea of the joint effect of the analytical errors and of the individual variability of the plants by comparing the analytical results from several specimens of the same plant species simultaneously cultivated in the same solution. Two such cases are reported in tables I and II. From them it is clear that a high degree of accuracy cannot be

TABLE I

ANALYSES OF FOUR SPECIMENS (A, B, C, D) OF NICOTIANA CULTIVATED IN SOLUTION IV. MILLIGRAM EQUIVALENTS PER KILOGRAM OF DRIED PLANT SUBSTANCE

SPECIMEN	LI	NA	K	MG	CA	MN
A	101	20		475	800	
B	95	20	1700	510	605	5.0
C	109	34	1960	590	660	5.2
D	100	27	1800	530	635	6.1
Mean	101	25	1820	540	700	5.4

TABLE II

ANALYSES OF FOUR SPECIMENS (A, B, C, D) OF SINAPIS CULTIVATED IN SOLUTION V. MILLIGRAM EQUIVALENTS PER KILOGRAM OF DRIED PLANT SUBSTANCE

SPECIMEN	NA	K	RB	CS	MG	CA	SR
A	430	1700	77	92	500	540	340
B	590	1420	88	91	440	640	540
C	850	1340	77	86	610	700	505
D	590	1560	91	96	560	660	550
Mean	615	1510	83	91	520	640	480

claimed. All conclusions from the analytical data obtained must thus be drawn with due allowance for the variability of the plant material as well as considerable analytical errors. Nevertheless, as will be seen from the following sections, several distinct rules concerning cation absorption by the plants studied can certainly be established.

In many cases the roots of the plants grown in the same culture vessel were found to be so interwoven with each other that it would have been very difficult to disentangle them. In such cases one had to analyze the plants without roots. It may thus be questioned whether the data obtained in this manner are at all representative of the total cation content of the plant and hence of its cation selectivity. In order to answer this question some analyses of detached roots and shoots were made. These showed, in agreement with the corresponding results of BURSTRÖM (5), that: the equivalent percentages of K, Rb, and Cs were roughly the same in the roots as in the shoots; that the percentages of Li, Ca, and Sr were lower in the roots than

in the shoots; and finally that the percentages of Mg, and especially those of Na, Mn, and Cu, were higher in the roots than in the shoots. Of the total cation content of the plant on the average only about $\frac{1}{10}$ was found in the roots. From a closer examination of these data, which are not given here in detail, one may infer that analyses of the shoots alone give a rather satisfactory picture of the cation content of the entire plants except that the Na content is perhaps sometimes found to be slightly too small and that the Mn and Cu contents may possibly be far too small. It seems possible, however, that the major part of the last two mentioned cations has been in some way precipitated on the root surface or in the cell walls of the roots without being truly absorbed by the living parts of the plant.

The culture experiments with solution I were performed in 1935; with solution II, partly in 1936 and partly in 1938; with solutions III, IV, and V in 1937. Temperature, light, and other conditions were uncontrolled and, therefore, different in the different years.

Analytical results

The main body of analytical data is presented in tables III to VII. Some of the figures given represent the average of 2 to 4 samples analyzed.

The analytical results were originally calculated so as to indicate the cation content in milligram equivalents per kilogram dry weight of plant material. The figures calculated in this way are, of course, influenced by the varying accumulation of starch, cellulose, and other organic substances in the plant. In order to avoid this, most analytical data are here given in another form: the sum of equivalents of all cations found in one kilogram dry weight was calculated and the amount of each cation expressed as percentage of this sum. In tables III to V, VII, and VIII all values for cations are expressed in this way. In tables VI and X this was not possible and the cation contents in these tables are therefore given as milligram equivalents per kilogram dry weight. In all tables under the head "total" the sum of the milligram equivalents found in one kilogram dry plant material is given. In each series the values for two plants with maximum cation content are printed with heavy faced type and those for two plants with minimum cation content are in italics.

Specific differences concerning the cation selection

The magnitude and specific character of the differences

An inspection of tables III to VII reveals that the different species vary in their capacity for selective accumulation of cations; but it is also seen that the amplitude of the specific variations is very different in regard to the different cations.

The greatest variations were met in the case of sodium. Thus, of the

plants cultivated in solution I (table III) the one richest in Na had a relative Na content (percentage of total cations) 32 times greater than that of the plant poorest in Na. With solution II (table IV) an even greater difference was encountered. The maximum Na percentage was 56 times greater than the minimum one. The corresponding ratios $Na_{max.} : Na_{min.}$ were in the case of the plants cultivated in solution III, 29 (table V); solution IV, 86 (table VI); and solution V, 19 (table VII). It is easy to convince oneself

TABLE III

ANALYSES OF ENTIRE PLANTS CULTIVATED IN SOLUTION I

Species	Equivalent percentages†					
	Na	K	Mg	Ca	Sr	Total‡
Atriplex hortense	**19.7**§	*39*§	**31**	*10*	*0.0037*	**4780**
A. litorale	10.7	56	23	*10*	0.0058	4340
Avena	3.7	**73**	14	*8*	*0.0037*	*2040*
Fagopyrum	*0.9*	*39*	27	**33**	**0.0107**	3230
Helianthus	*2.3*	54	17	27	0.0083	3020
Nicotiana	4.0	51	24	21	0.0056	4440
Pisum	6.0	62	*12*	20	0.0080	*2140*
*Plantago lanceolata**	12.2	45	18	24	0.0101	3690
*P. maritima**	**28.5**	*39*	*11*	21		4370
Solanum	4.1	44	25	27	0.0104	4290
Spinacia	4.5	52	31	13	0.0061	**6520**
Vicia	10.6	44	19	26	**0.0105**	2470
Zea	2.9	**70**	16	11	0.0052	2420
Culture solution	25.0	25	25	25	0.0062	

* Both species of Plantago were cultivated in pure silica sand irrigated with solution I. Tops were used for analyses.

† The sum of equivalents of all cations found in one kilogram dry weight was calculated and the amount of each cation expressed as percentage of this sum.

‡ In all tables under the head "Total" the sum of the milligram-equivalents found in one kilogram dry plant material is given.

§ In each series, the values for two plants with maximum cation content are printed with heavy faced type and those for two plants with minimum cation content are printed in italics.

of the truly specific character of these variations. In spite of the fact that the culture experiment analyses were performed in different years, we see that certain plant species (*Atriplex hortense, A. litorale, Plantago maritima,* Salicornia, Sinapis) are invariably characterized by a high Na content; certain other species (Fagopyrum, Zea, Helianthus) are as constantly distinguished by a remarkably low Na content. These specific differences in the absorption of Na remain essentially unaltered irrespective of the fact that the Na content of the culture solution was in some experiments rather high (25 per cent. of the sum of all cation equivalents in solution I) and in others very low (only 0.08 per cent. for solution III).

Towards the other alkali cations the different species behave much more uniformly. Thus, the highest relative potassium content of the plants

TABLE IV

ANALYSES OF PLANTS (WITHOUT ROOTS) CULTIVATED IN SOLUTION II

SPECIES	EQUIVALENT PERCENTAGE							
	NA	K	RB	MG	CA	SR	MN	TOTAL
Aster	5.2	27	30	*14*	23	.	0.22	3060
Atriplex hortense	**27.9**	12	14	32	15	*0.014*	0.36	4900
A. litorale	10.3	*11*	20	31	28	0.018	0.36	**5160**
Avena	4.7	25	**41**	15	*14*	*0.013*	0.44	2620
Chenopodium	0.9	23	27	28	21	0.016	0.18	4390
Fagopyrum	0.5	19	18	39	23	0.019	0.53	3320
Helianthus	*0.7*	24	29	21	25	0.021	0.39	3770
Lactuca	5.7	24	34	*13*	22	0.015	**1.19**	3620
Melilotus	8.4	22	25	19	25		0.22	2270
Nicotiana	3.5	**28**	28	22	19	0.024	*0.07*	3550
Papaver	5.4	25	**37**	18	16	*0.014*	0.15	2970
Pisum	1.4	23	27	18	32	0.024	0.49	2150
Plantago lanceolata	6.9	22	30	19	22	0.025	0.12	3460
P. maritima	**16.3**	12	*12*	23	37	**0.030**	0.23	3130
Salicornia	12.5	*11*	*13*	**50**	*14*	0.016	*0.04*	**8230**
Salsola	1.3	16	15	**40**	27		0.18	3540
Sinapis	10.9	18	26	18	30	**0.035**	0.26	3650
Solanum	3.5	15	22	25	33	0.022	0.51	3360
Spinacia	4.6	23	24	33	15	0.016	0.41	4860
Vicia	8.6	22	28	16	25	0.021	**0.57**	2060
Zea	*0.7*	**31**	28	29	*13*	.. ..	0.29	*2220*
Culture solution	20	20	20	20	20	?	0.10	.

cultivated in solution I was only 1.9 times greater than the lowest one. In the plants cultivated in the other solutions the corresponding ratios ($K_{max.} : K_{min.}$) were, respectively, 2.8 (solution II), 2.3 (solution III), and 1.8 (solution V), *i.e.*, always of a quite different order of magnitude than in the case of Na. It could now perhaps be supposed that the relative constancy of the K content would be connected in some way with the fact that

TABLE V

ANALYSES OF PLANTS (WITHOUT ROOTS) CULTIVATED IN SOLUTION III

SPECIES	EQUIVALENT PERCENTAGE						
	NA	K	MG	CA	SR	MN	TOTAL
Atriplex hortense	**2.31**	47	32	18	0.054	0.37	**4450**
Avena	0.32	**75**	16	*8*	*0.025*	0.58	2320
Fagopyrum	*0.08*	*32*	**42**	24	0.073	0.46	3480
Helianthus	*0.09*	45	25	29	0.083	0.68	4070
Lactuca	0.38	**69**	*13*	16	0.040	**0.89**	2820
Pisum	0.25	55	13	31	0.054	**1.62**	*1540*
Plantago maritima	**0.66**	*35*	24	39	**0.103**	*0.30*	3690
Solanum	0.21	45	26	29	**0.070**	*0.11*	4010
Spinacia	0.14	55	32	*13*	*0.026*	0.37	**5090**
Vicia	..	61	*13*	25	0.052	0.33	*2120*
Culture solution	0.08	33	33	33	0.08	0.08	....

TABLE VI

ANALYSES OF PLANTS (WITHOUT ROOTS) CULTIVATED IN SOLUTION IV. MILLIGRAM EQUIVALENTS PER KILOGRAM OF DRY WEIGHT

SPECIES	LI	NA	MG	CA
Atriplex hortense	103	475	>475	398
Avena	55	20	280	*170*
Fagopyrum	*49*	*16*	>500	560
Helianthus	108	17	>475	800
Melilotus	75	21	450	625
Nicotiana	101	25	540	700
Pisum	50	29	250	245
Salsola	66	78	...	460
Sinapis	100	140	>475	>875
Vicia	79	83	315	365
Zea	*30*	*5.5*	430	*130*

TABLE VII

ANALYSES OF PLANTS (WITHOUT ROOTS) CULTIVATED IN SOLUTION V

SPECIES	EQUIVALENT PERCENTAGE							
	Na	K	Rb	Cs	Mg	Ca	Sr	TOTAL
Atriplex hortense	24.1	*37*	*1.6*	*1.0*	27	*5.7*	*3.9*	5970
Avena	3.4	61	3.3	1.7	14	10.9	5.4	2760
Fagopyrum	*0.4*	*37*	*1.6*	*1.0*	24	21.2	13.7	4660
Helianthus	1.6	49	2.5	1.5	20	14.8	10.5	3140
Melilotus	3.1	47	2.1	2.0	*13*	19.2	13.3	4050
Nicotiana	4.2	53	2.1	2.0	17	10.5	10.3	4280
Pisum	1.9	56	3.3	2.5	*12*	12.6	11.4	*1660*
Salsola	3.5	49	2.4	2.3	21	12.7	8.7	5760
Sinapis	15.6	38	2.1	2.3	13	15.7	12.2	3940
Vicia	28.6	*33*	2.3	2.2	17	8.6	7.3	2200
Zea	*1.3*	67	3.1	2.1	15	*6.4*	5.0	2020
Culture solution	42	42	2.1	2.1	4.2	4.2	4.2	

TABLE VIII

ANALYSES OF PLANTS (WITHOUT ROOTS) CULTIVATED IN SOIL

SPECIES	EQUIVALENT PERCENTAGE					
	Na	K	Mg	Ca	Sr	TOTAL
Atriplex	28.0	35	24	13	0.019	3300
Helianthus	0.1	65	10	26	0.029	2930
Nicotiana	0.1	46	10	43	0.047	3030
Pisum	0.4	37	11	53	0.057	1730

K is an element essential for plant growth while Na is unessential. But that such an assumption would not be correct is shown by the fact that the elements Rb and Cs, though as unessential as Na, nevertheless show about the same range in this respect as does K. Thus, the ratio $Rb_{max.} : Rb_{min.}$ was, for

the plants cultivated in solution II, 3.4, and in solution V, 2.1; whereas the ratio $Cs_{max} : Cs_{min}$ was found to be 2.5 in the case of the plants from solution V, which was the only solution containing this element. In spite of the fact that the differences in percentages of total base present as K, Rb, and Cs are relatively small it seems rather obvious that they are not merely due to analytical errors or accident; an inspection of the analytical data shows that there are some species (Avena, Helianthus, Pisum,[2] Spinacia, and Zea) which were always relatively rich in K, Rb, and Cs, while certain other species (*Atriplex hortense, Plantago maritima,* and Fagopyrum) were always found to be relatively poor in these elements. Such differences may therefore be designated as specific, at least for the most part.

In view of the old experience that there are certain "lithium plants" characterized by their great capacity to accumulate this element, one would perhaps expect a very large amplitude of variation in the absorption of this element, especially since the collection of plant species cultivated by us includes such a notorious lithium plant as Nicotiana. According to our experimental results, however, the variation of Li content is not excessive; the maximum relative Li content is only 3.6 times greater than the minimum. It should, however, be noted that we have not had opportunity to observe the most extreme cases of Li accumulation; according to TSCHERMAK (quoted by VON LINSTOW) there are plants (Cirsium) which accumulate Li to a distinctly greater degree than does Nicotiana.

The magnesium, calcium, and strontium contents also show a variability of moderate amplitude. Thus, the ratio $Mg_{max} : Mg_{min}$ was in the case of the plants cultivated in solution I, 2.8; in solution II, 3.8; in solution III, 3.2; and in solution V, 2.2. Though the Mg determinations, due to technical difficulties, were in general less accurate than perhaps any others, it is clear that these differences are, to a considerable extent, specific in their nature. Thus all Chenopodiacean plants (Atriplex, Spinacia, Salicornia, Salsola) and also Fagopyrum were always rich in this element; Pisum, Vicia, and Avena were, on the other hand, relatively poor in it.

The ratio $Ca_{max} : Ca_{min}$ was respectively, 4.1 (plants cultivated in solution I); 2.8 (solution II); 4.9 (solution III); 6.7 (solution IV); and 3.7 (solution V). The variations of relative Sr content were of about the same magnitude. The ratio $Sr_{max} : Sr_{min}$ was 2.9 in the plants of solution I; 2.7 in those of solution II; 4.0 in those of solution III; and 3.5 in the plants of solution V. The specific character of these differences is rather obvious. Avena, Zea, and Spinacia are constantly low in both Ca and Sr; Fagopyrum, *Plantago maritima,* Helianthus, and Sinapis absorb these elements relatively copiously.

[2] The total cation content of Pisum (per unit of dry matter) was always rather low. Its absolute K content is, therefore, not very high (7, fig. 1) even when the relative percentage of K is considerable.

It is thus evident that among the alkali and earth alkali cations Na is the cation which responds most to specific variations in the plants. This has, in fact, been observed already by VAN ITALLIE (**13**). In the cell sap of different species of Characeae, Na was also found to show a wider amplitude of specific variation than the other cations studied (**6**). It is not at all clear, however, why even Na should behave in such a peculiar fashion.

The manganese content of the plants also exhibits a high degree of variation. Thus the ratio $Mn_{max.} : Mn_{min}$ amounts to 30 for plants cultivated in solution II and to 15 for plants in solution III; but contrary to all the elements so far discussed, the Mn content varies rather irregularly. The cause of these irregularities are not known with certainty. It seems conceivable, however, that it may be found in an occasional precipitation of some slightly soluble Mn compounds. It should also be noted that, due to the exceptionally high Mn content of many roots, the Mn absorption of the plants shows a very different aspect depending on whether the whole plant or only its aerial parts are analyzed.

The total sum of cation equivalents per unit of dry matter is also a distinctly specific character. Thus, for example, Atriplex, Salicornia, and Spinacia are invariably distinguished by an unusually great total of cation equivalents; Avena, Pisum, Vicia, and Zea, on the contrary, by a relatively small total. Since the first named plants are relatively high in water, however, their cation concentrations would appear lower if the fresh weight or the water content of the plants was chosen as a basis for the calculation instead of their dry matter.

THE SPECIFIC CATION SELECTION AS CORRELATED WITH THE ECOLOGICAL AND TAXONOMICAL CHARACTER OF THE PLANT

As already mentioned, *Salicornia herbacea, Plantago maritima, Atriplex hortense,* and *A. litorale* are among the species found to absorb the greatest amount of Na. This indicates that there exists a positive correlation between a strong absorption capacity for Na (or perhaps more correctly stated: a want of exclusion power against Na) on the one hand, and the halophytic character of the plant on the other. (*A. hortense,* though now a garden plant, was probably originally a halophyte). This rule is, however, not free from exceptions. *Aster tripolium,* though a typical halophyte, showed in our experiments a lower Na content than many non-halophytic plants; *e.g., Sinapis alba,* and *Vicia sativa.*[3] On the other hand, the low Na content of *Salsola kali* apparently makes an exception; the variety of Salsola cultivated by us turned out to be not the one which occurs on seashores but var. *tenui-*

[3] It should, however, be mentioned that Aster has been until now cultivated only once, in an experimental work; its cation selection has not been as thoroughly studied as that of most other species.

folia which is known to occur on landing places and rubbish heaps. It is thus no true halophyte.

It has been known for a long time that halophytes when growing on their natural saline substrates contain much more Na and Cl than do glycophytes growing on their natural non-saline substrates. In more recent years it has also been shown that halophytes absorb more Cl even from non-saline soils than do glycophytes from the same soil (**23**). As far as the present writer is aware, however, it has not been definitely established until now (**22**) that halophytes, when several alkali cations are equally available, nevertheless absorb relatively much more Na than do glycophytes under the same conditions. Probably this specific lack of power to exclude Na ions is a character which in some way enables the halophytes to thrive on saline substrates not suited to requirements of the glycophytes. The relations between salt selection and halophytic character is the subject of other investigations in this laboratory and the question will not now be discussed.

The data obtained in the present investigation are too incomplete to give a reliable picture of the correlation existing between the taxonomic position of a given plant species and the peculiarities of its cation selection. Only a few suggestions connected with this matter can be pointed out here.

Perhaps the most striking observation of this kind is the remarkably high Mg content found in all Chenopodiacean plants analyzed, *viz., Atriplex hortense, A. litorale,* Chenopodium, Salicornia, Salsola, and Spinacia. On the other hand the high Na content which is characteristic of the Chenopodiaceans (**4**) was lacking in the non-halophytic members of this family.

Further, it could be pointed out that both of the Graminae analyzed (Avena and Zea) are characterized by their low Ca and Sr content, a feature already known (**4**). Also, the three Leguminosae studied (Melilotus, Pisum, and Vicia) show a general resemblance in their cation absorption although the absence of marked peculiarities makes it difficult to state this resemblance more positively. Finally, the two species of Atriplex show an obvious similarity in their cation selection. On the other hand the two species of Plantago studied could—in spite of some character common to both of them—easily be distinguished on account of their different selection alone.

Validity of the above mentioned rules under other growth conditions

It has already been pointed out that in spite of the fact that culture experiments were performed during the course of several years and that the external conditions (temperature, etc.) were not constant, the specific differences in cation selection were essentially constant. Nevertheless, it seemed questionable if these specific characters would persist when the growth conditions were more profoundly changed; *e.g.*, when the plants were grown

not in water culture, as in the experiments so far reported, but in ordinary soil. To test this question, four plant species (*Atriplex hortense,* Helianthus, Nicotiana, and Pisum) were cultivated in a pot filled with ordinary garden soil and watered with tap water. The results of the analyses are given in table VIII. It is easily seen that the four plant species, also when cultivated in soil, have maintained most of those cation selection peculiarities which we already know from the water culture experiments:

1. The Na content exhibits a much greater degree of variability than that of any other cation studied.
2. Atriplex is by far the richest in Na of the four species studied. The difference in this respect between Atriplex and the three other species is even markedly greater for plants cultivated in soil than for those grown in water culture. Atriplex contained about 200 to 300 times more Na than Helianthus and Nicotiana whereas corresponding difference in the water culture experiments was generally almost ten times smaller.
3. Among the four plants studied, Atriplex had the smallest percentage of K.
4. Atriplex had by far the highest Mg percentage.
5. Atriplex had distinctly the lowest Ca and Sr percentages.
6. The Ca and Sr contents of the four plants varied in a strictly parallel manner.
7. Atriplex had the highest, and Pisum the lowest, total equivalents of cations per unit of dry matter.

As compared with these concordances between the results of the soil and the water culture experiments the discordances are relatively slight; the K percentage of Pisum in the soil culture was found to be unusually low and, above all, the Ca and Sr content of Helianthus in the soil culture was decidedly too small as compared with that of the other plants. Discrepancies, especially as to elements like Ca and Sr, are in fact easily conceivable. These elements occur in the soil not only as constituents of the soil solution, but also in an adsorbed state and probably also as slightly soluble solid salts ($CaCO_3$, etc.); the absorption of these salts is dependent on the chemical disintegrating power of the roots which in water culture experiments plays no rôle at all.

In this connection, some experiments carried out by other investigators deserve mention. NEWTON (16) grew six crop plants in a nutrient solution containing 4.7 milligram equivalents per liter of K, 4.6 of Mg, and 8.0 of Ca. Among the plant species studied were *Helianthus annuus, Pisum sativum,* and *Zea mays.* The difference between the cation contents of these plants was slight with the exception of Zea. It was found to contain 3.0 times less Ca than Pisum and 4.3 times less Ca than Helianthus. Our own results are quite in accord with these observations.

BERTRAND and GHITESCU (**2**) cultivated four plant species side by side in a garden. Among them were *Avena sativa* and *Fagopyrum esculentum*. It was found that Fagopyrum contained, per unit of dry matter, 1.5 times more K, 3.7 times more Mg, and 4.3 times more Ca (but 51 times less Na) than did Avena. For the sake of comparison it may be stated that of our plants cultivated in solution II, Fagopyrum contained almost as much K, 3.3 times more Mg, and 2.1 times more Ca (but 7.2 times less Na) than did Avena. The general trend of the results in both experiments is thus undoubtedly the same although the differences as to Na are decidedly more pronounced among the plants of BERTRAND and GHITESCU than among ours.

Finally, VAN ITALLIE (**13**) cultivated eight species of phanerogams in pots filled with three kinds of soil: (a), a poor sandy soil; (b), the same soil with some Na_2SO_4 added; and (c), the first mentioned soil with Na_2SO_4 and K_2SO_4 added. Among the plants studied were *Avena sativa* and *Sinapis alba* (white mustard). From the data given by VAN ITALLIE it may be calculated that the plants contained approximately the relative cation percentages indicated in table IX. These figures show that Avena had absorbed about 2 to 3 times less Na and Ca, but in most cases about twice as much K, as had

TABLE IX

APPROXIMATE CATION PERCENTAGES OF AVENA AND SINAPIS CULTIVATED BY VAN ITALLIE IN THREE DIFFERENT KINDS OF SOIL (A, B, C)

PLANT	Na			K			Mg			Ca		
	A	B	C	A	B	C	A	B	C	A	B	C
Avena	5	17	11	68	61	66	11	10	10	16	12	13
Sinapis	14	26	19	32	28	41	14	14	11	40	34	29

Sinapis; the Mg percentages were almost the same in both plants. On the other hand, in our own experiments with the solutions II and V (tables IV and VII), Avena had taken up 2 to 3 times less Na, about 2 times less Ca, about 1½ to 2 times more K, and about as much Mg, as did Sinapis. The agreement between the principal results of VAN ITALLIE and ours is thus very good.

Summing up the results of the above comparisons of plants cultivated under different conditions, we may conclude that the main differences in cation selection between our plant species grown in water culture are by no means restricted to the special conditions prevailing in our experiments. They are, at least to a considerable extent, of a general nature. On the other hand, it has been clearly demonstrated (**21**) that the external conditions do definitely affect the cation selection of higher plants.

Features common to the cation selectivity of all the plants studied

From emphasis upon the differences between various plant species, we now put the question: which features are common to the cation selectivity of all the plants?

CONSTANT RELATIONSHIPS BETWEEN CATIONS

As a first constant feature we may point out the striking fact that all of our experimental plants absorb rubidium very nearly with the same rapidity as potassium, from a solution containing equivalent amounts of these cations.

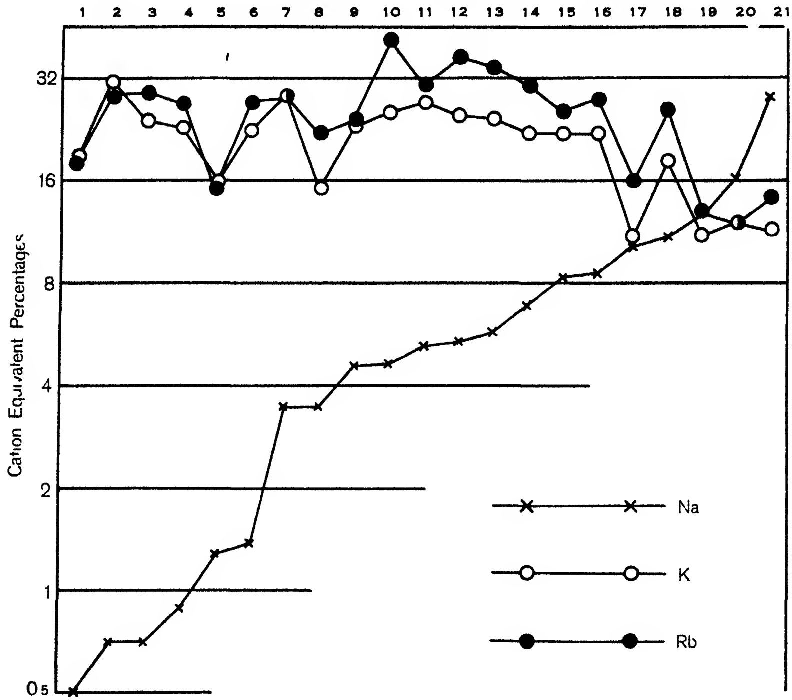

FIG. 1. Equivalent percentage of Na, K, and Rb in plants cultivated in solution II containing these cations in equivalent amounts. The plants are arranged according to increasing Na content.

1. Fagopyrum
2. Zea
3. Helianthus
4. Chenopodium
5. Salsola
6. Pisum
7. Nicotiana
8. Solanum
9. Spinacia
10. Avena
11. Aster
12. Papaver
13. Lactuca
14. *Plantago lanceolata*
15. Melilotus
16. Vicia
17. *Atriplex litorale*
18. Sinapis
19. Salicornia
20. *Plantago maritima*
21. *Atriplex hortense*

This is clearly seen from table IV and figure 1. According to the results, the ratio $K_{absorbed}:Rb_{absorbed}$ varies only between the values 1.0:1.88 (*Atriplex litorale*) on the one side and 1.1:1.0 (Fagopyrum, Salsola, Zea) on the other. It is not possible to decide from the data of table IV, whether the apparent variations of this ratio are real or perhaps due only to analytical errors. That the ratio between K and Rb absorbed is at least approximately constant is remarkable.

In the experiment with solution V (table VII), which contained a 20 times lower concentration of Rb than of K, the amounts of Rb taken up by the plants were all about 20 times smaller than the corresponding amounts of K. At first sight this seems strange since ions are generally taken up relatively more abundantly from dilute solutions than from those more concentrated. An explanation of this result, however, will be given later in this paper.

A second characteristic common to all of the plants studied is that they absorb cesium nearly as rapidly as rubidium, and thus also as rapidly as potassium (table VII). According to the analytical results the ratio $Rb_{absorbed}:Cs_{absorbed}$ varies between 1.9:1.0 (Avena) on the one side and 1.0:1.1 (Sinapis) on the other; the average is about 1.3:1.0. In this case it is questionable whether the ratio in reality varies slightly or is constant.

While the last three members of the alkali-metal series show such a close resemblance in their behavior towards the plants it is rather remarkable that between the first three members of this group (Li, Na, and K) no correlation can be detected; that is, apart from the fact that the plants richest in Na contain a slightly lower percentage of K than do most other species.

A third characteristic common to all of the plants studied is that they take up strontium at almost the same rate as calcium; the analytically determined ratio $Ca_{absorbed}:Sr_{absorbed}$ varies in the experiment with solution V (table VII) between 2.0:1.0 and 1.0:1.0. Again these variations do not exceed the limits of experimental error.

If the nutrient solution contains Ca and Sr in different concentrations, each of these cations is taken up in amounts directly proportional to the concentration of that ion in the solution. The cation occurring in a lower concentration is thus in this case *not* absorbed relatively more abundantly. This is clearly seen from figure 2 which refers to the experiment carried out with solution III. This solution contained Sr in a concentration 400 times lower than the concentration of Ca and the graph shows that the amounts of Sr taken up by the plants are nearly exactly 400 times smaller than the amounts of Ca absorbed by the same plants. It is also striking to see how the Ca and Sr curves fluctuate in parallel. This indicates that the absorption power of a given plant species for Sr is always proportional to the absorption power of the same species for Ca. The data obtained with solu-

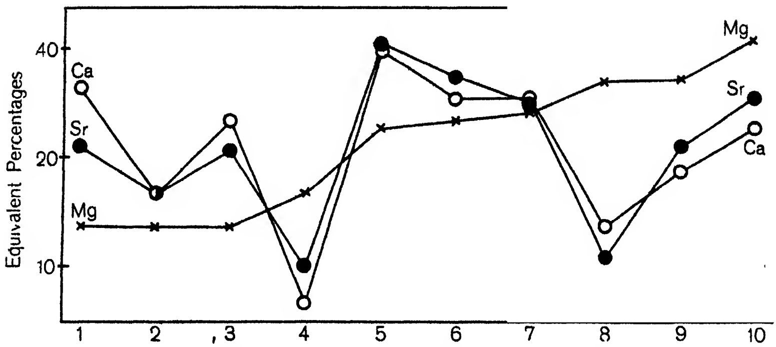

FIG. 2. Equivalent percentage of Mg, Ca, and Sr in plants cultivated in solution III containing, among other constituents, 4 milligram-equivalents of Mg and Ca and also 0.01 milligram-equivalents of Sr per liter. The Sr percentages are represented 400 times magnified. Plants are arranged according to increasing Mg content.

1. Pisum
2. Lactuca
3. Vicia
4. Avena
5. *Plantago maritima*
6. Helianthus
7. Solanum
8. Spinacia
9. *Atriplex hortense*
10. Fagopyrum

tions I, II, and IV would, if represented graphically, give very similar curves. In all cases the relative Mg content varies independently of the relative Ca and Sr content or at least nearly so.[4]

OTHER CATIONS

It might be conceivable that a given cation (A) would be absorbed by all plants in constant ratio to another—say five or ten times more slowly than another cation (B). Such a case would in itself not be more surprising than our finding that Rb and Cs are, in all plant species studied, absorbed with almost the same rapidity as K, or that Sr is taken up by all plants almost as rapidly as Ca. It is, therefore, a remarkable and rather unexpected fact that those ionic ratios, which are constant for different plants, are equal to, or at least approximate to, unity; *i.e.*, the above named ratios $K_{absorbed} : Rb_{absorbed} : Cs_{absorbed}$ and $Ca_{absorbed} : Sr_{absorbed}$, seem to be the only ones that are independent of the specific nature of the plant. All other ionic ratios which deviate from unity are also very variable from species to species. The explanation is not quite clear, but perhaps it may be found in the assumption that the plants are unable to "distinguish" between K, Rb, and Cs and also between Ca and Sr.

[4] In a preliminary account (7) it was claimed that there also exists a positive correlation between the Mg and Ca contents, although some exceptions were obvious. This statement was based on a comparison of the absolute Mg and Ca contents which show, in fact, a rough parallelism. This parallelism is, however, mainly attributable to the fact that there are some plants which are poor in salts and others that are rich in them.

The variation in most ionic absorption ratios from species to species is so great that it is impossible to arrange even the four cations most closely studied (Na, K, Mg, and Ca) in a constant series according to decreasing absorption. Thus in table III the series K > Ca > Mg > Na, and K > Mg > Ca > Na both occur in several plants; but also the series K > Mg > Na > Ca (Atriplex), and K > Na > Ca > Mg (*Plantago maritima* have been encountered. Table IV shows an even more complex situation: from solution II sometimes K or Rb, sometimes Mg, sometimes Ca, and sometimes even Na is taken up most copiously; in solution II, however, just as in solution I, the cations Na, K, Mg, Ca, and also Rb occur in equivalent amounts. This striking difference between the behavior of the plants in solution I and II seems to be due to a peculiar kind of mutual competition between K and Rb ions; this will be more closely examined later. These interactions between different cations make it still more difficult, of course, to put forth any series of general applicability for cation absorption.

In spite of these complications we shall now try to find from the analytical data contained in tables III to VII some more or less general rules concerning the absorption of the different cations. In doing this we restrict our comparisons to those cations which are present, in the same culture solution, in equivalent concentrations.

Lithium.—Earlier literature statements (**4**, **14**) about "lithium plants" easily give the impression that this element would be accumulated to a very high degree by some plants. As far as I am aware there are, however, no previous culture experiments carried out with solutions of known Li concentrations which permit a quantitative estimation of the actual Li accumulation by any plant. The experimental results obtained in the present investigation show that, although different plant species behave differently towards Li ions, this element always belongs to those which are absorbed in relatively small amounts. Thus the eleven plant species cultivated in solution IV (table VI) which contained Li, Na, Mg, and Ca in equivalent amounts all absorbed much less Li than either Mg or Ca; and thus also much less K or Rb. Remarkably enough this is even true of such a typical (though not extreme) lithium plant as Nicotiana. On the other hand, the majority of the new plant species studied took up somewhat more Li than Na.

It is conceivable that Li would be accumulated to a relatively greater extent when present in very low concentration in the nutrient solution. Even in this case, however, the accumulation is apparently moderate since all of the plants cultivated in solution I were found to contain more Mn than Li, with the single exception of Nicotiana which had absorbed about twice as much Li as Mn. This solution contained about 0.005 milligram equivalents of Li and Mn per liter in addition to other ions.

Sodium.—As previously pointed out, the extent to which this element is

absorbed varies very much depending upon the specific nature of the plant in question. Nevertheless, Na seems to be the one least absorbed of all the cations studied or, in other words, the one most perfectly excluded. Perhaps the most striking example of the exclusion of Na is afforded by Fagopyrum cultivated in solution V (table VII). From this solution (containing in addition to other ions, 2 milligram equivalents of Na and K, and 0.2 milligram-equivalents Mg and Ca) Fagopyrum was found to have taken up about 90 times less Na than K, and about 5 to 6 times less Na than Mg or Ca.

Na is in most cases absorbed less, often much more, than K, Rb, Ca, Mg, Ca, and Sr.[5] In many cases the absorption of Na is even less than that of Li or Mn.

POTASSIUM AND RUBIDIUM.—These two cations are, in most cases, more easily absorbed than any others. Thus, if Rb is not present, K is nearly always[6] taken up in greater amounts than any other cation studied (tables III, V). Even when the absorption of K is suppressed by the presence of an equal amount of Rb (solution II), no other cations are accumulated much more than K and Rb.

CESIUM.—This cation seems to be absorbed almost as easily as Rb and thus about as easily as K.

MAGNESIUM, CALCIUM, AND STRONTIUM.—Sr is absorbed, by all of the plants studied, almost as readily as Ca. Mg, on the other hand, is absorbed to concentrations 5 times as great by some species and by others only to concentrations $\frac{1}{3}$ of those of Ca and Sr. Generally speaking the alkaline earths are accumulated in quantities which amount to about 1/9 to 1/1 of the simultaneously absorbed quantities of K (tables III, V) if the absorption of K is not suppressed by Rb. In the presence of an equivalent amount of Rb, however, the alkaline earths are often taken up to even a greater degree than is K.

MANGANESE.—The plants cultivated in solution III, which contained equivalent amounts of Mn and Sr, were always found to have absorbed distinctly more Mn than Sr; the amounts of Mn found in the shoots for the most part surpass their Sr content by about 10 to 20 times (table V). In spite of this, it seems very questionable whether it would be justifiable to conclude that Mn is in general more easily absorbed than Sr. It should be noted that in the above experiment the absorption of Sr has probably been strongly sup-

[5] From solution III (see table V) containing Na and Sr in equivalent amounts, Na was taken up as copiously or in most cases even more copiously than Sr, but this result is probably due to the "antagonistic" effect of Ca. This element occurs in the culture solution in a concentration 400 times higher than that of Sr and thus may be able to suppress the absorption of Sr very strongly.

[6] The only exception from this rule is Fagopyrum which, when cultivated in solution III, contains a little more Mg than K (table V). In view of the relatively low accuracy of the Mg determinations it is, however, questionable whether this single exception is a real one.

pressed by the Ca ions, the concentration of which in the culture solution III was 400 times higher than that of the Sr ions.

A comparison of the quantities of Mn and Na absorbed from solution III is probably more justifiable. The ratio $Mn_{absorbed} : Na_{absorbed}$ varies between about 8:1 (Helianthus) and 1:6 (Atriplex). This indicates that on the average Mn is taken up to about as small a degree as is Na.

Finally, it should be stressed once more that the roots contain relatively large quantities of Mn which have been left out of account in the above considerations. If the Mn content of the roots is included, the absorption of Mn appears considerably greater.

COPPER.—Cu is an example of a heavy metal which in very low concentrations can easily be determined in the flame spectrum. A culture experiment was started with solution VI containing besides other ions, Cu, Mn, and Sr in equivalent amounts (0.05 milligram-equivalents per liter). From the results given in table X it is clear that the amounts of Cu transferred from the roots to the upper parts of the plant are very small; in fact, considerably

TABLE X

Cu, Mn, AND Sr CONTENT OF (A) ROOTS AND (B) AERIAL PARTS OF PLANTS CULTIVATED IN SOLUTION VI. THE CATION CONTENTS ARE EXPRESSED AS MILLIGRAM EQUIVALENTS PER KILOGRAM DRY WEIGHT

SPECIES	Cu		Mn		Sr	
	A	B	A	B	A	B
Avena	100	<3.8	13	19	6	8
Helianthus	20	3.8	5	18	3	10
Pisum	190	<3.8	>175	15	14	8

smaller than the corresponding amounts of Mn and Sr. In the roots, on the other hand, great amounts of Cu were found. It is questionable, however, whether these quantities were really absorbed by living cells or only precipitated on or in the cell walls of the roots.

CATION ABSORPTION SERIES

After this review of the absorption of single cations we may suitably return to the question of the arrangement of the cations studied in a series, according to decreasing absorption by the plants. It was pointed out that it is not possible to put forth such a single series valid for all of the plant species studied. If we, however, intentionally leave the less frequent types of cation selection out of account, it is possible to lay down a cation series roughly valid for some sort of average plant type. This series, which is here given only with explicit reservation for its limited validity, may be written as follows:

$$\text{Rb, K, Cs} > \begin{matrix}\text{Mg}\\ \text{Ca,}\end{matrix}\ \text{Sr} > \begin{matrix}\text{Li}\\ \text{Na}\\ \text{Mn}\end{matrix}$$

Elements separated by a comma differ only slightly from each other as to their absorption. On the other hand, the symbols written above one another indicate elements the relative absorption of which is subject to variations so great that it would seem too arbitrary to give precedence to one of them.

The present writer knows no other experiments in which higher plants have been cultivated in solutions containing a considerable number of cations in equivalent concentrations and in which the amounts of different cations absorbed by the plants have been determined. It is, therefore, scarcely possible to compare the results arrived at in this investigation with the results of previous work by other investigators. Only in passing it may be pointed out that the above cation series agrees approximately, but not wholly, with that obtained in cultivating the alga *Tolypellopsis stelligera* in a nutrient solution, containing several cations in equivalent amounts (**8**). The cation series referring to Tolypellopsis is K > Rb > Ca > Na, Li. The only difference is that the alga absorbs somewhat less Rb than K. Another Characean alga, *Nitella clavata*, studied by HOAGLAND AND DAVIS (**10**) yielded the series K > Na > Ca > Mg. This cation series differs somewhat more from the series valid for the majority of phanerogams but coincides with that found in our experiments with *Plantago maritima.*

Some interactions between different cations in absorption

No attempt will be made here to discuss the intricate question regarding effects exerted by some cations on the absorption of others. Only a restricted but striking aspect of this problem will be considered.

It has already been shown that, in spite of the fact that the culture solutions I, II, and III all contained K, Ca, and Mg in equivalent amounts, all of the plants cultivated in solutions I and III absorbed distinctly more K than either Ca or Mg; the same plant species when cultivated in solution II were often found to contain more Mg or Ca than K. What is the cause of this apparent inconsistency? The most conspicuous difference between solutions I and III and solution II is that the first named solutions were free from Rb, while solution II contained as much Rb as K. It is possible, therefore, to explain the experimental results by assuming that Rb ions when present in the culture solution exert a specific depressing effect on the absorption of K ions.

If the above interpretation is correct it seems rather natural to expect that a similar effect would be obtainable with another pair of physicochemically allied cations (Ca and Sr). This is apparently the case, as may be concluded from the following chain of comparisons. From the analytical data presented in table VII it is obvious that Sr is absorbed almost as readily as Ca; from table IV it may be concluded that by almost all of the plant species represented Ca is absorbed distinctly more than Na. Hence, it seems

logical to conclude that Sr should be taken up by most plant species at least as much as Na. Contrary to this view, however, table V shows that all of the plants cultivated in solution III (which contained Na and Sr in equivalent amounts) have taken up much less (about 10 times less) Sr than Na. This result is easily explained by the previous assumption that the Ca ions, being present in solution III in a concentration 400 times higher than the concentration of Sr, have strongly depressed the simultaneous absorption of Sr.

In order to prove, once more, the above assumptions, the following experiments were carried out: A series of seven culture solutions (A to G) was prepared so comparisons could be drawn between selected pairs of cultures in which, with reference to any one of the 6 cations investigated, the concentration of one cation was varied by a tenfold change. Besides constant concentrations of NH_4NO_3 (2 milligram equivalents), $MgSO_4$ (0.2 milligram equivalents), Fe-tartrate, and the components of the "A–Z" solution of HOAGLAND, the solutions had the following cation composition with their anion contents held as constant as possible:

SOLUTION	CONCENTRATIONS EXPRESSED AS MILLIGRAM EQUIVALENTS PER LITER					
	LI	NA	K	RB	CA	SR
A	0.2	2.0	2.0	0.2	0.2	
B	*2.0*	2.0	2.0	0.2	0.2	
C	0.2	*0.2*	2.0	0.2	0.2	
D	0.2	2.0	*0.2*	0.2	0.2	
E		2.0	2.0	*2.0*	0.2	0.2
F		2.0	2.0	0.2	*2.0*	0.2
G		2.0	2.0	0.2	0.2	*0.02*

Avena, Helianthus, and Pisum were cultivated in these solutions. Figure 3 shows graphically the amounts of K, Rb, and Sr absorbed by the plants. These amounts are expressed as milligram equivalents per kilogram dry matter. An inspection of the graphs reveals the following facts: The Rb content of the plants is not influenced to any degree by variations of the Li, Na, Ca, and Sr concentrations of the culture solution; but a decrease of its K concentration from 2.0 to 0.2 milligram equivalents (solution D) causes a very conspicuous increase in the Rb absorption of all plants. As to the absorption of K, the result is correspondingly, though somewhat less, uniform; the K content of the plants is also rather independent of the Li, Na, Ca, and Sr concentrations of the culture solution. When, however, the concentration of the Rb ions in the solution is raised from 0.2 to 2.0 milligram equivalents (solution E) it causes a distinct depression of the K absorption in both Avena and Helianthus. This depression is, however, for some unknown reason not evident in Pisum. With regard to the modifica-

tion of the Sr absorption the three species studied again behave similarly: figure 3 shows that if the Ca concentration of the culture solution is raised from 0.2 to 2.0 milligram equivalents (and the Rb concentration simultaneously lowered from 2.0 to 0.2 milligram equivalents) the plants take up about 2 to 5 times less Sr.

It is thus evident that the absorption of Rb is more strongly depressed by K ions than by any other cation the concentration of which was varied in the above experiment. Correspondingly, the absorption of K is more strongly depressed by Rb ions than by any other cation studied. Finally, the absorption of Sr is suppressed more strongly by Ca ions than by any

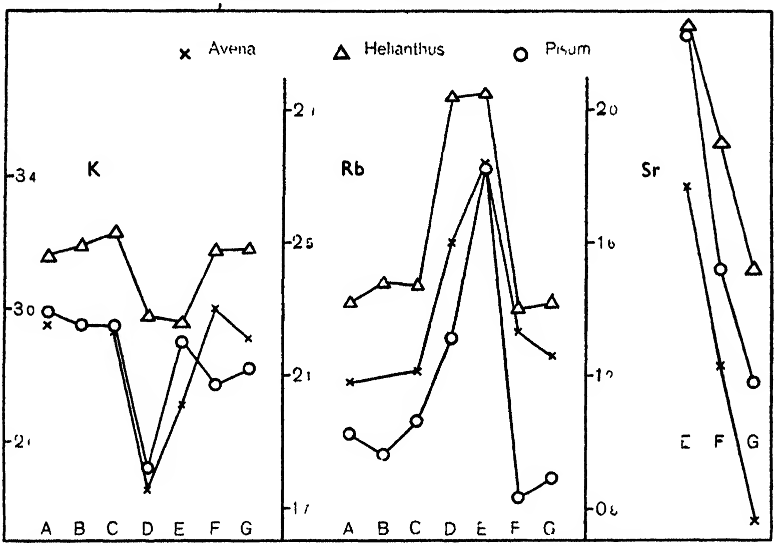

FIG. 3. K, Rb, and Sr content of Avena, Helianthus, and Pisum cultivated in the solutions A to G. The ordinates indicate logarithms of the cation contents expressed as milligram-equivalents per kilogram of dry matter.

other cations tried. Whether Sr correspondingly suppresses the absorption of Ca has not been experimentally decided but seems, by analogy, very likely.

It is found that the absorption of a certain kind of cation is most strongly depressed, not by cations of a pronouncedly different type (*e.g.*, the absorption of univalent ions by bivalent ions) but, on the contrary, by very closely allied cations. This might seem somewhat unexpected in view of some current conceptions of "ion antagonism." It was only in compiling this publication that the writer was made aware of the observations of HURD-KARRER (12) according to which K salts are able to prevent the harmful effect of Rb salts, and Ca salts the toxic action of Sr salts. These observations were explained by HURD-KARRER by assuming that Rb ions are replaced by K ions and Sr ions by Ca ions in the nutrition of the plants—an assumption which

in view of the present investigation turns out to be entirely correct. BLANCK (**3**) has also observed that Rb may compete with K absorption (**24**). Moreover, HOAGLAND, DAVIS, and HIBBARD (**11**) made an analogous observation concerning the accumulation of anions in establishing that the absorption of Br ions by Nitella cells is depressed by Cl and I ions but not by SO_4 or NO_3 ions. These authors pointed out that it may be questioned whether the expression "ion antagonism" should be used to designate phenomena of this type. The following considerations seem to stress these doubts and also shed some light on the mechanism of the mutual hindrance observed.

It is a well-known fact that ions are in general absorbed to relatively greater extent from dilute than from concentrated solutions (**6, 8, 11, 25**). Using the terminology of STILES and KIDD (**25**) we may also express this by saying that the absorption ratio (the ratio $c_{internal} : c_{external}$) of a given ion increases when its concentration in the nutrient solution decreases. Also the results obtained in the present investigation offered several examples of this. It may suffice to quote one single typical example. The ratio $K_{absorbed}$: $Na_{absorbed}$ was in the case of Avena cultivated in solutions I and V which both contained K and Na in equivalent amounts, respectively $73 : 3.7 = 20$ and $61 : 3.4 = 18$; hence in both samples almost the same. Solution III, on the other hand, contained Na in a concentration 400 times lower than K. If the amounts of Na absorbed by the plants were directly proportional to the concentration of Na in the nutrient solution, one would expect to find in the Avena plants cultivated in solution III a Na percentage about $400 \times 19 = 7600$ times smaller than the corresponding K percentage. But in reality the Na percentage analytically found (0.32) was not 7600 but only 230 times smaller than the K percentage (75). In other words, when the concentration of Na in the culture solution was reduced 400 times (in relation to K) the amount of Na absorbed decreased only 33 times. Na was thus absorbed relatively much more from the dilute solution.

Strangely enough, however, Rb in the presence of K, and Sr in the presence of Ca, are conspicuous exceptions to this general rule. Thus Rb, when occurring in the culture solution in the same concentration as K (solution II), was accumulated roughly to the same extent as K and when occurring in a concentration 20 times lower than K (solution V) was accumulated in amounts roughly 20 times smaller than the simultaneously absorbed amounts of K. The behavior of Sr as compared with Ca is quite analogous. When both cations are present in equivalent concentrations (solution V) Sr is absorbed almost as readily as Ca; when Sr is given in a concentration 400 times lower than Ca (solution III) it is absorbed in roughly 400 times smaller amounts than Ca. Even when the concentration of Sr is only about 1/4000 of that of Ca (solution I) the proportionality in absorption is at least approximately maintained; the absorbed amounts of Sr are about

1/4000 to 1/2000 of those of Ca. Hence, the relative enhancement of absorption which in other cases is brought about by diluting the salt solution is not found in these cases. Why? One answer to this question is, of course, that K when present in a much higher concentration than Rb strongly depresses the accumulation of this ion and that Ca correspondingly depresses the absorption of Sr. But this answer, though in itself quite correct, does not explain the peculiar fact that this depression is exactly great enough to maintain the absorption ratio of Rb (as compared with that of K) and of Sr (as compared with that of Ca) constant in spite of the great variations to which the concentrations of these ions are subjected in the nutrient solutions used. The simplest explanation of this unexpected constancy of the absorption ratios seems to be that the plants are in a certain sense unable to "distinguish" between K and Rb and also between Ca and Sr in the same manner as they are unable to distinguish between two different isotopes of a given element. Just as the absorption ratio of the radioactive isotope remains about the same as that of the inactive isotope, (even when the two isotopes occur in extremely different concentrations in the external medium) so it is also easy to understand that the absorption ratio of Rb or Sr remains approximately the same as that of K or Ca when the proportions K: Rb and Ca: Sr are subjected to great variations in the culture solution. We must assume, however, that the absorption ratio depends, not upon the individual concentrations of K, Rb, Ca, and Sr, but upon the combined concentrations of K + Rb in the one case and of Ca + Sr in the other.

From the same point of view it is easy to explain some peculiarities in the results obtained with the culture solutions A to G. On first sight it might appear strange that, as the K concentration of the solutions was reduced 10 times, the amount of K absorbed decreased only about 2 to 3 times. As the concentrations of Rb and Sr were also 10 times reduced, however, the amounts of these cations in the plants decreased about 4 to 10 times. This result is understandable if we take into consideration the following facts. In solution E the joint concentration of Ca + Sr amounts to 0.4 milligram equivalents and in solution G to 0.22; the difference is thus moderate. Similarly, the combined concentration of K + Rb in solution E (4.0 milligram equivalents) differs only moderately from that in D (2.2 milligram equivalents). In these instances, therefore, the reduction of the concentrations of Sr or Rb must evidently cause a considerable decrease of the amounts of these elements absorbed. When, however, we compare solution A which contains 2.2 milligram equivalents K + Rb with solution D which contains only 0.4 milligram equivalents K + Rb we find that the total sum of these cations is much reduced in solution D. It thus seems only natural that the absorption ratio of K, just like that of Rb, should be considerably greater in D than in A.

It is then somewhat inappropriate, if the views presented above are correct, to use the term "ion antagonism" for the phenomena discussed; "mutual competition" or "mutual replacement" are expressions which would give a clearer picture of the actual situation.

Concluding remarks

In the present investigation stress has been laid on obtaining positive experimental facts concerning the selective cation absorption of plants. Hitherto such facts have rarely been available. While it would be highly important to give a theoretically satisfactory explanation of the selective absorption process in question on the basis of the facts thus established, the present writer, unfortunately, feels unable to do this as the problem is too intricate. Only a few general remarks may be ventured here.

There are at least two different processes which may account for the entrance of salts from the nutrient solution into the plant. The first is the transpiration stream which will carry the salts contained in the nutrient solution into the plant insofar as the salts are not hindered by some semipermeable or selectively permeable structures in the roots. Since the cell walls are generally much more permeable to both water and salts than are the protoplasts, it seems conceivable, and perhaps even probable, that the transpiration stream traverses the root cortex; flowing not only through the protoplasts but to a considerable extent also between them—*i.e.*, through the cell walls which probably exert only a relatively slight selective effect. It is probable, however, that there is at least one point in which the stream is forced to pass through living protoplasts. This point is the endodermis, the Casparian strips of which make its cell walls more or less impermeable to water and salts (**19**). Hence, the composition of the salt mixture entering the plant with the transpiration stream will, in any case, be controlled by the permeability of some living protoplasts. The same is true in regard to the salts which tend to enter the central cylinder of the root by diffusion from the nutrient solution. It seems advisable to consider whether or not it might be possible to explain the cation selection exerted by the plants in the light of current theories of protoplasmic permeability.

The ultra-filter hypothesis is evidently not able to explain our experimental results. From what we know about membranes acting as ultra-filters (*e.g.*, collodion and copper ferrocyanide membranes) the bivalent Mg, Ca, and Sr ions should permeate with considerably greater difficulty than the univalent Na ions. In reality, as we have seen, Na is taken up to a less degree by most of our experimental plants than are the alkaline earth cations. This finding also proves that, contrary to a wide-spread opinion, the diffusibility (or mobility) of the ions is not, or at least not alone, decisive for their absorption by the plants. In this connection it may be noted that

from a purely physiological point of view the smallness of the Na absorption is perhaps somewhat surprising, since it is generally supposed that plant protoplasts are more permeable to Na than to Mg or Ca ions; plasmolytical experiments especially point in this direction. Either this supposition is wrong or the cation absorption of the plants is, to a considerable degree, determined by some factor other than protoplasmic permeability.

Turning then to the lipoid-solubility theory we note that at least in the system butyl alcohol/water, and thus probably in most systems of the type lipoid/water, the distribution coefficients of the cations decrease in the order: Li > Na > K > Rb > Mg > Ca > Sr [ALLEMANN, (1) and unpublished results]. This is, of course, not at all in harmony with the cation selection observed in the present investigation. However, according to OSTERHOUT, *et al.* (**17**) the relative solubilities in guaiacol increase in order Li < Na < K < Rb < Cs, which would be at least roughly in accordance with the behavior of the roots; but Mg and Ca are stated (**18**) to be much less soluble in this solvent than Na. This is again in contradiction to the selection exerted by most roots. We must bear in mind that the distribution of cations between lipoidal solvents and water is still very imperfectly known; it is thus possible that in the future lipoids may be found whose solvent capacity would better satisfy the theoretical demands now in question. At any rate it is so far quite uncertain that it will be possible to bring the cation selection of the plants even into purely formal agreement with the lipoid-solubility theory; not to speak of the possibility of explaining the cation selection in a truly plausible manner.

There is, too, no clear evidence that the combined lipoid-filter theory, though making many different cation selection series theoretically conceivable, would be able to give a really credible explanation of the whole process.

It is in fact scarcely worth while to speculate very much upon the concordances and discrepancies between the current permeability theories and the cation selection exerted by the roots. From other investigations, especially of HOAGLAND and his collaborators (**9**) it is rather evident that this process is due principally to a complicated activity of the root cells. This is essentially independent of the transpiration stream and it cannot be deduced from our knowledge of the passive permeability of the protoplasts. This activity presents itself most obviously in the ability of the root cells to perform work in accumulating salts in high concentration from the very dilute solution surrounding them. The root cells probably actively secrete the salts taken up, releasing them to the xylem vessels in which they are conducted with the ascending sap to the shoot.

The problem of the salt selection of the higher plants is thus in the first place a question of the selectivity of the adenoid (active) salt transport of the root cells. There are, however, still other complications not yet men-

tioned. Thus, for example, some of the cations transported with the transpiration stream to the shoot are probably sent back along the sieve tubes to the roots where they are likely to impede, at least to some extent, the further absorption of cations of the same kind while other sorts of cations are retained in the shoot and thus exercise no repressing effect on the uptake of cations of their own kind.

It is evident that the problem of cation selection of the higher plants is an extremely intricate one.

Summary

Some 20 phanerogams representing different ecological types were cultivated in complete nutrient solutions containing several cations in equivalent amounts. After approximately two months of growth the cation composition of the plants was determined using LUNDEGÅRDH's method of quantitative spectral analysis. The cations most closely studied in this way were Na, K, Rb, Mg, Ca, Sr, and Mn. Li and Cs were also studied but less extensively; Cu only quite cursorily. The results obtained were as follows:

1. The differences between the plant species cultivated in a given solution are very unequal in regard to the different cations. They are very great in the case of Na and Mn contents; the maximum values are about 20 to 60 times greater than the minimum. They are considerable in the case of the Li, Mg, Ca, and Sr contents; the maximum values are about 3 to 5 times greater than the minimum. For K, Rb, and Cs, on the other hand, only moderate differences were observed; the maximum values were 2 to 3 times greater than the minimum. The cause of variation of different cations in such unequal magnitudes is not known.

2. The differences observed are, for the most part, truly specific in character. Single plant species are constantly (irrespective of the year of cultivation and composition of the culture solutions) found to be relatively rich in certain cations and other species as constantly relatively rich in other cations.

3. All halophytes cultivated (*Salicornia herbacea, Plantago maritima, Atriplex litorale, A. hortense*) were found to be very rich in Na, with the single exception of *Aster tripolium* which contained only moderate quantities of this element. *Fagopyrum esculentum, Zea mays,* and *Helianthus annuus* were, on the contrary, distinguished by their unusually large exclusion of Na. All Chenopodiacean plants, as well as Fagopyrum, were remarkably rich in Mg. Ca and Sr were absorbed in greatest amounts by Helianthus, Fagopyrum, and *Sinapis alba;* Avena, Zea, and Spinacia absorbed these two cations in the least amounts.

4. All plants studied absorb Rb and Cs with almost the same rapidity as K. They also take up Sr almost as readily as Ca. On the other hand

all the other cations studied show no distinct mutual correlation in their absorption rates by the plants.

5. The specific differences between the absorption characteristics of different plant species are so great that it is not possible to arrange the absorption of the cations studied in any certain order which would be valid for all plant species investigated. The following data are, however, valid for the majority of the species studied. K, Rb, and Cs are, in general, the cations most copiously accumulated. The next place is in most cases occupied either by Ca and Sr or, about as frequently, by Mg, Li, Na, and Mn; the latter were taken up by most plant species to a distinctly less extent than all of the six cations so far mentioned. The absorption of Na amounted generally to about 1/50 to 1/2 of the simultaneously absorbed K.

6. K and Rb ions and Ca and Sr ions depress the absorption of each other more than do any other cations studied in this respect. It is also remarkable that Rb or Sr, in the presence of an excess of K or Ca, respectively, do not obey the general rule that the absorption ratio of a given ion increases when the concentration of that ion in the medium decreases. On the contrary, the absorption ratios of Rb and Sr remain constant if only the total concentration of K + Rb, respective to Ca + Sr, is held constant. These findings are explained by assuming that the ions K and Rb, and Ca and Sr, behave in the salt absorption of plants somewhat as identical ions or as two isotopes of the same element.

7. The very complex nature of the selective salt absorption of higher plants is stressed.

The author is indebted to Mr. Antti Kytöniemi, Mr. Leo Leiitoranta, and Mr. Mauri Siivonen for carrying out the spectral analyses on which this investigation is based. Mr. Kytöniemi also cultivated the plants grown in solution.

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INFLUENCE OF BORDEAUX MIXTURE AND ITS COMPONENT PARTS ON TRANSPIRATION AND APPARENT PHOTOSYNTHESIS OF APPLE LEAVES[1]

FRANKLIN W. SOUTHWICK AND N. F. CHILDERS

(WITH FOUR FIGURES)

Introduction

Spray materials should be judged not merely as an effective control against insects and pathogens but also by their immediate or residual influence on the treated plants; for it follows that those spray ingredients which are toxic to various insects and pathogens are in all probability toxic in some degree to plant protoplasm. The problem is to determine what effects, deleterious or otherwise, Bordeaux mixture and its component parts exert on the metabolism of apple leaves. Since this material is popular in the control of bitter rot, blotch, apple scab, fire blight, and other important fruit diseases, some knowledge concerning the influence of this spray on apple foliage would be of value in analyzing orchard spray problems.

The rates of transpiration and apparent photosynthesis of treated leaves were measured under different conditions of temperature, light, humidity, and soil moisture. These determinations along with other minor investigations were designed not only to obtain direct evidence as to the effect of this material on these processes but also to endeavor to provide some information as to the mechanism involved.

A concise review of most of the literature on the effects of Bordeaux mixture on plant metabolism has been assembled by MILLER (**23**).

Methods

The carbon dioxide gas analysis and procedure for measuring photosynthesis were similar to those described by HEINICKE and HOFFMAN (**12**). This apparatus was set up in connection with an environmental-control chamber as described by CHILDERS and BRODY (**5**) in which light, temperature, and humidity could be controlled. The temperature and humidity within the chamber were kept constant within a range of 2° F. and 2 per cent. relative humidity, respectively, unless otherwise stated. The humidity when not automatically controlled was recorded on a humidigraph inside the experimental chamber (on one occasion when the humidistat was out of order there were variations in relative humidity between five and ten per cent.). From these daily records, the average relative humidity for the duration of each experiment was determined and the vapor pressure calculated (**21**). The

[1] An abstract of this paper has been published elsewhere (**25**).

intensity of light reaching the experimental leaves was measured with a Weston photometer. The individual light readings were averaged for the check and test leaves.

Transpiration was measured simultaneously with photosynthesis by attaching to the air lines bottles which contained pumice stones impregnated with sulphuric acid (**11**).

Daily determinations which usually lasted three hours each were made between 7:30 A.M. and 7:00 P.M. on leaves of Stayman Winesap shoots. The shoots were from two to four feet long, arising from two- and three-year-old cutbacks and grown in the greenhouse in tubs which contained a dark clay loam soil. The trees in all experiments with the exception of those employed in experiment XII, had not set terminal buds. The data with a few exceptions represent an average of six treated leaves and an average of six untreated leaves evenly distributed up and down, and around shoots of one, two, or three trees. The number of trees used was dependent on the vigor of the shoots available per tree and the number of desirable leaves per shoot. Care was taken to see that the check and test leaves corresponded as to age and position on the shoots. The tree or trees involved in these experiments were watered regularly to the field capacity of the soil, except for the drought conditions study, at intervals ranging from once every day to once every sixth day, depending on the rate of transpiration and the evaporation from the soil as influenced by temperature and humidity in the chamber.

The Bordeaux mixture was prepared by first diluting the stock suspension of fresh hydrated lime and then adding the stock solution of copper sulphate according to PICKERING and BEDFORD (**24**). In all cases the sprays were applied immediately after they were prepared with a non-continuous hand atomizer, sometime between 6:00 and 10:00 P.M.

In some experiments, after the spray had been in contact with the leaf surface for several days, the spray sediment was removed, as much as possible, from either the upper or lower surface separately or both surfaces simultaneously. This was done by gently wiping the leaf surface with absorbent cotton moistened in distilled water. Extreme care was taken during this operation to prevent mechanical injury to the leaves. Spray removal was done sometime between 6:00 and 10:00 P.M.

The term "expected rate" refers to the relationship existing between the rate of photosynthesis and transpiration of the check and test leaves prior to spray treatment. It was assumed that the relationship between the check and the test leaves would remain constant throughout the experiment, within the limits observed prior to spraying, and that any further fluctuations arising in these relationships after spray was applied were due to the influence of the spray material. Consequently, the "percentage of expected rate" as used in this paper is considered to represent a measure of the effect

of various spray materials on the rate of photosynthesis and transpiration. The methods used in calculating the "expected rate" and the "percentage of expected rate" are given at the bottom of table I.

TABLE I

THE INFLUENCE OF BORDEAUX MIXTURE 4–6–100 ON THE RATE OF PHOTOSYNTHESIS AND TRANSPIRATION OF STAYMAN APPLE LEAVES
TEMPERATURE—50° F.
AVERAGE FOOT CANDLES ON CHECK LEAVES—1547
AVERAGE FOOT CANDLES ON TEST LEAVES—1302
AVERAGE VAPOR PRESSURE—4.95 MM. OF Hg

DATE 1939	APPARENT PHOTOSYNTHESIS				TRANSPIRATION			
	CO_2/100 CM.^{2}/HOUR		EXPECTED RATE $\frac{A \times 100}{B}$	PERCENTAGE OF EXPECTED RATE $\frac{C \times 100}{94^*}$	H_2O/100 CM.^{2}/HOUR		EXPECTED RATE $\frac{E \times 100}{F}$	PERCENTAGE OF EXPECTED RATE $\frac{G \times 100}{88^*}$
	TEST	CHECK			TEST	CHECK		
	A	B	C	D	E	F	G	H
	mg.	*mg.*		%	*gm.*	*gm.*		%
Nov. 14	16.52	17.18	96	102	0.72	0.86	84	95
15	12.67	13.04	97	103	0.98	1.02	96	109
16								
17								
18	14.38	15.95	90	96	0.96	1.15	83	94
Average			94*	100			88*	100
1st spray 19	10.47	11.85	88	94	1.01	1.23	82	93
20	14.70	16.97	87	93	0.81	0.90	90	102
21	12.36	13.99	88	94	1.12	1.15	97	110
2nd spray 22	11.95	16.11	74	79	1.00	1.18	85	97
23	11.23	14.31	78	83	0.67	0.81	83	94
24	10.99	15.35	72	77	1.18	1.23	96	109
25	12.34	15.87	78	83	0.38	0.43	88	100
3rd spray 26								
27	10.42	13.82	75	80	0.77	0.87	89	101
28	12.27	17.80	69	73	0.82	0.98	84	95
29	14.06	18.84	75	80	1.01	1.11	91	103
30	10.67	14.09	76	81	0.86	0.99	87	99
Dec. 1†	8.67	12.24	71	76	0.73	1.03	71	81
2	20.30	24.63	82	87	0.74	0.91	81	92
3‡								
4	10.94	13.77	79	84	0.93	1.15	81	92
5	14.50	19.69	74	79	0.85	0.98	87	99

* The numbers that are starred in columns C and G represent the "expected rate" and are substituted in the formula above columns D and H obtaining the "percentage of expected rate" in columns D and H.
† Spray removed from upper surface.
‡ Spray removed from lower surface.

Results

It has been a general observation that cool temperatures and moist conditions, such as occur in early spring, are closely associated with Bordeaux

injury to apple leaves. In the summer, however, when the temperatures are considerably higher, little or no visible injury ordinarily develops. Consequently, the first five experiments to be discussed were designed to determine what rôle various temperature levels, ranging from 50 to 100° F. play in affecting the rate of photosynthesis and transpiration of apple leaves sprayed with 4–6–100 Bordeaux. It may be pointed out that variations in photosynthesis and transpiration of the test leaves (expressed in percentage of expected rate) before the spray applications were made are considered in each experiment to be normal for that set of leaves and such variations after treatment are likewise considered within normal range of fluctuation. To gain an average estimate of the effect of the spray on leaf activity, an imaginary curve could be projected through the daily percentage of expected rate of photosynthesis or transpiration.

Experiment I (temp. 50° F.)

Two Stayman Winesap trees each bearing a single vigorous shoot were employed in this experiment. A relationship was established between the check and test leaves, in the five-day period from November 14 to 18. On the evenings of November 18, 21, and 25 a spray of Bordeaux 4–6–100 was applied to both surfaces of the test leaves. The data are given in table I and graphically shown in figure 1–A as the percentage of expected rate for the sprayed leaves. The rate of photosynthesis following the first spray application was consistently below 100 per cent. of the expected rate but this reduction was hardly significant. Immediately following the second spray, however, a decrease of 21 per cent. in the photosynthetic rate was apparent. The rate of carbon dioxide absorption remained at about this level even after the third spray. On the evening of November 30 the spray deposit was carefully wiped from the upper surface of the test leaves with moist absorbent cotton. On the morning of December 1, approximately 12 hours following spray removal, some burning typical of the Bordeaux injury described by Hedrick (10), Crandall (7), and Dutton (9) was observed on the test leaves. The injury appeared as spots scattered over the upper surface and were most numerous at the tip and margins of the test leaves. Contrary to the reports of the above workers, the mature leaves were more seriously affected than the young leaves. Two days later, on the night of December 2, the spray residue on the lower surface of the treated leaves was removed. No further increase in injury was observed. To be sure that this injury was not due to the wiping action alone, the check leaves were wiped similarly with moist absorbent cotton. No visible injury ensued.

It is apparent from figure 1–A that the rate of photosynthesis was not changed appreciably at 50° F. by spray removal from either the upper or lower leaf surface. Transpiration which heretofore was not significantly

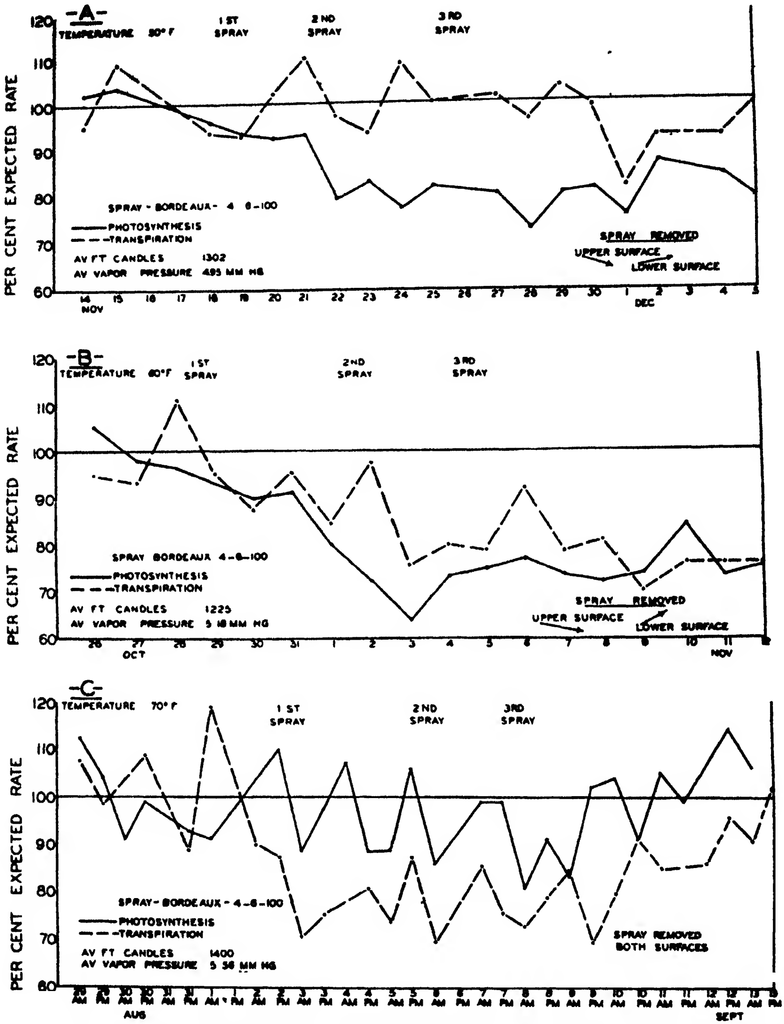

FIG. 1. The effect of Bordeaux mixture 4–6–100 on the rate of photosynthesis and transpiration of apple leaves at (A), 50° F.; (B), 60° F.; and (C), 70° F.

influenced by Bordeaux mixture dropped sharply immediately after the removal of the spray material from the upper surface and at the time the visible injury occurred. By December 5, however, three days after the removal of the spray deposit from the lower surface, transpiration had recovered.

EXPERIMENT II (TEMP. 60° F.)

The data for this experiment are given in table II and presented graphically in figure 1–B. Each of the two Stayman apple trees used possessed

TABLE II

THE INFLUENCE OF BORDEAUX MIXTURE 4–6–100 ON THE RATE OF PHOTOSYNTHESIS AND TRANSPIRATION OF STAYMAN APPLE LEAVES

TEMPERATURE—60° F.
AVERAGE FOOT CANDLES ON CHECK LEAVES—2083
AVERAGE FOOT CANDLES ON TEST LEAVES—1225
AVERAGE VAPOR PRESSURE—5.18 MM. OF Hg

DATE 1939		APPARENT PHOTOSYNTHESIS				TRANSPIRATION			
		CO_2/100 CM.^{2}/ HOUR		EXPECTED RATE $\frac{A \times 100}{B}$	PERCENTAGE OF EXPECTED RATE $\frac{C \times 100}{121^*}$	H_2O/100 CM.^{2}/ HOUR		EXPECTED RATE $\frac{E \times 100}{F}$	PERCENTAGE OF EXPECTED RATE $\frac{G \times 100}{111^*}$
		TEST	CHECK			TEST	CHECK		
		A	B	C	D	E	F	G	H
		mg.	mg.		%	gm.	gm.		%
Oct.	26	26.34	20.81	127	105	1.12	1.06	106	95
	27	22.20	18.63	119	98	1.49	1.45	103	93
	28	28.36	24.39	116	96	1.37	1.11	123	111
Average				121*	100			111*	100
1st spray	29					1.61	1.51	107	96
	30	18.68	17.14	109	90	1.07	1.10	97	87
	31	25.13	22.88	110	91	1.07	1.00	107	96
Nov.	1	9.11	9.38	97	80	1.37	1.48	93	84
2nd spray	2	15.00	17.24	87	72	1.39	1.27	109	98
	3	20.36	26.10	78	64	1.07	1.27	84	76
	4	14.61	16.55	88	73	1.15	1.29	89	80
3rd spray	5	24.64	27.03	91	75	1.12	1.27	88	79
	6	29.14	31.15	94	78	1.20	1.18	102	92
	7	31.09	34.71	90	74	1.15	1.30	88	79
	8†	18.04	20.74	87	72	1.13	1.26	90	81
	9	17.60	19.81	89	74	0.89	1.12	79	71
	10‡	19.10	18.79	102	84	1.21	1.40	86	77
	11	17.88	20.16	89	74	1.91	2.25	85	77
	12	17.66	19.36	91	75	1.38	1.60	86	77

* See table I.
† Spray removed from upper surface.
‡ Spray removed from lower surface.

one shoot which had not set a terminal bud. A relationship was established between the check and test leaves in a three day period from October 26 to 28. The 4–6–100 Bordeaux spray was applied during the early evening of October 28, November 1, and November 4; and the spray residue was removed the nights of November 7 and 9 from the upper and lower leaf surfaces, respectively. As shown in figure 1–B, the photosynthesis curve of the test leaves in percentage of expected rate was very similar to the one obtained in experiment I and shown in figure 1–A. The sharpest drop in

photosynthesis during this experiment occurred, as in the previous test, after the second spray application; for on November 3 the rate of carbon dioxide absorption of the sprayed leaves was reduced 36 per cent. The rate of photosynthesis rose slightly November 4 but still remained 25 per cent. below the expected rate, where it persisted for the duration of the experiment. In contrast to the practically negligible influence of Bordeaux 4–6–100 on transpiration in experiment I, the rate of water-vapor loss was definitely reduced, 24 per cent. by November 3, two days after the second spray had been applied. The transpiration rate continued to show a definite reduction thereafter, with an exception on November 6, when it partially recovered.

There was no visible injury noted on the test leaves before spray removal. When, however, the spray was carefully removed on November 7 from the upper surface of the test leaves with moist absorbent cotton, the same type of injury was visible the following morning as appeared after similar treatment in experiment I conducted at 50° F. All of the check leaves were similarly wiped but in no case did any injury appear on them. Here again the injury was more pronounced on the mature leaves. Photosynthesis failed to recover following the spray removal on November 8 from the upper surface or after November 9 when the spray deposit was removed from the lower surface. The data in table II and figure 1–B show that the rate of transpiration, however, was reduced even farther following the appearance of injury on the foliage.

The results of this experiment indicate a definite reduction in both photosynthesis and transpiration following applications of Bordeaux mixture 4–6–100. These reductions persisted even though the spray sediment was eventually removed. As in experiment I, the operation of spray removal brought about the appearance of visible injury apparently instigated by Bordeaux mixture but made visible only after wiping the upper surface of the test leaves with moist absorbent cotton.

Experiment III (temp. 70° F.)

In order to determine the critical temperature for the appearance of injury following spray removal, as shown in experiments I and II, the temperature for this experiment was raised to 70° F. where it was held constant throughout the experiment. The two Stayman Winesap apple trees, each of which bore a single shoot, had not set terminal buds. Since one of the test leaves was broken midway through the experiment, the data in table III and figure 1–C represent the average of only five test leaves, and the usual six check leaves. A relationship between the check and test leaves was established from August 29 to September 2. Three applications of a 4–6–100 Bordeaux were made on the evenings of August 2, 5, and 7, then

TABLE III

THE INFLUENCE OF BORDEAUX MIXTURE 4–6–100 ON THE RATE OF PHOTOSYNTHESIS AND TRANSPIRATION OF STAYMAN APPLE LEAVES

TEMPERATURE—70° F.
AVERAGE FOOT CANDLES ON CHECK LEAVES—1220
AVERAGE FOOT CANDLES ON TEST LEAVES—1400
AVERAGE VAPOR PRESSURE—5.56 MM. OF Hg

DATE 1939	APPARENT PHOTOSYNTHESIS				TRANSPIRATION			
	CO_2/100 CM.^{2}/HOUR		EXPECTED RATE $\frac{A \times 100}{B}$	PERCENTAGE OF EXPECTED RATE $\frac{C \times 100}{148^*}$	H_2O/100 CM.^{2}/HOUR		EXPECTED RATE $\frac{E \times 100}{F}$	PERCENTAGE OF EXPECTED RATE $\frac{G \times 100}{166^*}$
	TEST	CHECK			TEST	CHECK		
	A	B	C	D	E	F	G	H
	mg.	*mg.*		%	*gm.*	*gm.*		%
Aug. 29 A.M.	25.77	15.55	166	112	1.93	1.55	125	108
29 P.M.	19.65	12.75	154	104	.....	..	.....	.....
30 A.M.	19.69	14.68	134	91	1.43	1.24	115	99
30 P.M.	22.38	15.36	146	99	1.96	1.56	126	109
31 A.M.	.....	.....	.....	.....	.....	.....	.....	.....
31 P.M.	19.04	14.15	135	91	1.26	1.22	103	89
Sept. 1 A.M.	23.10	17.10	135	91	1.10	0.80	138	119
1 P.M.	.....	.....	.....	.....	1.00	0.96	104	90
2 P.M.	20.23	12.80	163	110	2.09	2.04	102	88
Average			148*	100			116*	100
3 A.M.	22.69	17.22	132	89	0.91	1.11	82	71
3 P.M.	15.44	10.71	144	97	1.98	2.26	88	76
1st 4 A.M.	20.29	12.83	158	107	.....	.....	.....	.....
spray 4 P.M.	28.04	21.39	131	89	1.82	1.93	94	81
5 A.M.	23.20	17.57	132	89	0.70	0.81	86	74
5 P.M.	19.21	12.21	157	106	1.66	1.62	102	88
6 A.M.	28.82	22.49	128	86	0.81	1.00	81	70
2nd 6 P.M.	.....	.....	.....	.....	.....	.....	.....	.....
spray 7 A.M.	25.39	17.41	146	99	0.85	0.85	100	86
7 P.M.	24.06	16.49	146	99	1.76	2.00	88	76
8 A.M.	25.78	21.83	118	80	0.75	0.88	85	73
8 P.M.	21.09	15.74	134	91	1.44	1.57	92	79
3rd 9 A.M.	23.14	18.88	123	83	0.69	0.71	97	84
spray 9 P.M.	19.57	13.12	149	101	1.41	1.74	81	70
10 A.M.	25.97	16.82	154	104	1.86	2.00	93	80
10 P.M.	21.42	15.87	135	91	1.27	1.20	106	91
11 A.M.†	22.80	14.70	155	105	2.08	2.10	99	85
11 P.M.	20.22	13.74	147	99	.....	.....	.....	.....
12 A.M.	.....	.....	.....	.....	1.99	1.99	100	86
12 P.M.	15.81	9.40	168	114	1.97	1.77	111	96
13 A.M.	26.54	16.71	159	107	1.70	1.61	106	91
13 P.M.	17.08	13.18	130	88	1.64	1.38	119	103

* See table I.
† Spray removed from both surfaces.

subsequently removed from both surfaces the night of September 10. Owing to the rather wide fluctuations in the daily percentage of expected

rates following applications of Bordeaux, no conclusive statement from this experiment may be made as to the effect of this spray on photosynthesis. Yet there seems to be little doubt that the trend in photosynthesis of the test leaves was downward following applications of Bordeaux 4–6–100. The fact that the rate of carbon dioxide absorption was, for the most part, above the expected rate after spray removal would indicate that this operation caused a recovery in photosynthesis, which is brought out more clearly in later experiments. No visible injury could be observed either before or after spray removal.

Transpiration was noticeably decreased by treatment with Bordeaux mixture immediately after the first application when the rate was reduced 29 per cent. The rate of water-vapor loss failed to recover to its pre-spray status until three days following removal of the spray deposit from both surfaces.

The results of this experiment indicate perhaps a slight reduction in photosynthesis when compared with the reductions obtained in photosynthesis in experiments I and II. Transpiration, however, dropped more sharply after spraying in this investigation at 70° F. than in those at 50° and 60° F. discussed previously.

Experiment IV (temp. 82° F.)

Bordeaux is frequently applied in some sections of the country in the cover sprays for apple trees without the appearance of injury to either fruit or foliage. In an effort to determine the effect of Bordeaux 4–6–100 on apple foliage under summer conditions, the temperature within the environmental-control chamber was held constant at 82° F. Owing to a sudden unexpected "burning out" of about half of the twenty-four 1000-watt light bulbs in the control chamber during this experiment, the light intensity on the test leaves averaged only 525 foot candles.

The data presented in table IV and figure 2–D represent two daily 2½-hour determinations from six test and six check leaves selected from two vigorous Stayman apple trees. After a pre-spray relationship was established from September 16 to 19, Bordeaux 4–6–100 was applied to both surfaces of the test leaves on the evenings of September 19, 22, and 25. Then on September 29 as much of the spray residue as possible was removed with moist absorbent cotton. According to figure 2–D the rate of photosynthesis appeared to be reduced slightly by two spray treatments. On the morning following the third spray application, however, the rate of carbon dioxide absorption dropped sharply to 19 per cent. below the expected rate. This reduced rate of photosynthesis persisted until September 30, the day after spray removal. At this time the relative rate of carbon dioxide absorption gradually rose until October 2 when photosynthesis approximately regained its pre-spray status.

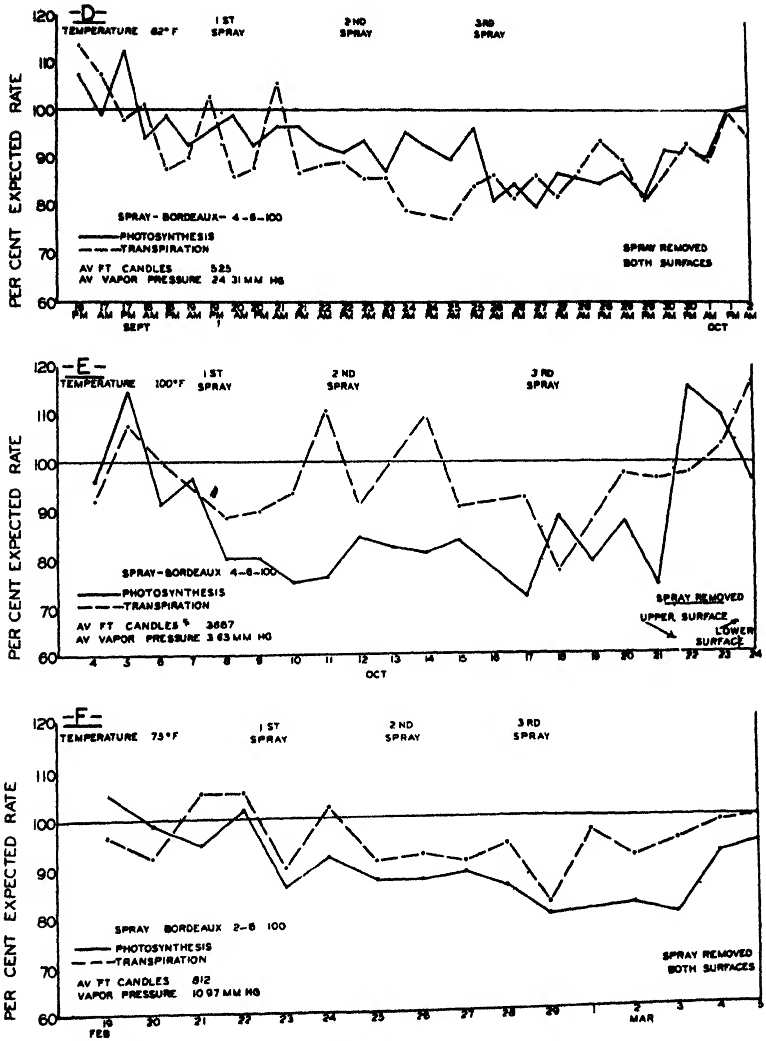

FIG. 2. The effect of Bordeaux mixture 4–6–100 at (D), 82° F., and (E), 100° F., on the rate of photosynthesis and transpiration of apple leaves. The lower graph (F) shows the influence of Bordeaux mixture 2–6–100 at 75° F. on leaf activity.

Except on the morning of September 21, transpiration appeared to be reduced by applications of Bordeaux 4–6–100. The maximum reduction in the rate of water-vapor loss amounted to 23 per cent. the morning of September 25, three days after the second spray application. The rate of transpiration never fully recovered until October 1, two days after the spray had been removed from both surfaces of the test leaves.

TABLE IV

THE INFLUENCE OF BORDEAUX MIXTURE 4–6–100 ON THE RATE OF PHOTOSYNTHESIS AND TRANSPIRATION OF STAYMAN APPLE LEAVES

TEMPERATURE—82° F.
AVERAGE FOOT CANDLES ON CHECK LEAVES—341
AVERAGE FOOT CANDLES ON TEST LEAVES—525
AVERAGE VAPOR PRESSURE—24.31 MM. OF Hg

DATE 1939	APPARENT PHOTOSYNTHESIS				TRANSPIRATION			
	CO_2/100 CM.²/ HOUR		EXPECTED RATE A × 100 / B	PERCENTAGE OF EXPECTED RATE C × 100 / 144*	H_2O/100 CM.²/ HOUR		EXPECTED RATE E × 100 / F	PERCENTAGE OF EXPECTED RATE G × 100 / 145*
	TEST	CHECK			TEST	CHECK		
	A	B	C	D	E	F	G	H
	mg.	*mg.*		%	*gm.*	*gm.*		%
Sept. 16 P.M.	17.66	11.30	156	108	2.91	1.77	164	113
17 A.M.	15.28	10.79	142	99	3.20	2.04	157	108
17 P.M.	16.28	10.12	161	112	2.78	1.96	142	98
18 A.M.	19.67	14.49	136	94	4.08	2.78	147	101
18 P.M.	21.08	14.78	143	99	3.41	2.73	125	86
19 A.M.	26.89	20.48	131	91	3.44	2.65	130	90
19 P.M.	17.93	12.96	138	96	2.55	1.70	150	103
Average			144*	100			145*	100
20 A.M.	20.83	14.59	143	99	3.22	2.57	125	86
20 P.M.	21.69	16.41	132	92	2.19	1.73	127	88
1st 21 A.M.	19.84	14.15	140	97	3.99	2.59	154	106
spray 21 P.M.	17.52	12.52	140	97	2.32	1.86	125	87
22 A.M.	17.35	12.94	134	93	3.30	2.06	127	88
22 P.M.	11.80	8.98	131	91	2.75	2.14	129	89
23 A.M.	19.10	14.13	135	94	3.36	2.72	124	86
23 P.M.	14.77	11.76	126	88	2.98	2.41	124	86
2nd 24 A.M.	16.54	11.98	138	96	3.00	2.62	115	79
spray 24 P.M.	16.68	12.58	133	92				
25 A.M.	15.23	11.83	129	90	3.26	2.90	112	77
25 P.M.	11.00	7.85	140	97	2.25	1.85	122	84
26 A.M.	13.26	11.42	116	81	3.76	2.99	126	87
26 P.M.	18.51	15.11	123	85	2.51	2.11	119	82
27 A.M.	13.16	11.43	115	80	1.76	1.38	128	88
3rd 27 P.M.	15.66	12.32	127	88	1.51	1.27	119	82
spray 28 A.M.					4.15	3.25	128	88
28 P.M.	16.20	13.20	123	85	2.38	1.75	136	94
29 A.M.	15.89	12.72	125	87	2.89	2.22	130	90
29 P.M.	14.32	12.08	119	83	2.09	1.75	119	82
30 A.M.	24.36	18.51	132	92	3.18	2.35	135	93
30 P.M.					2.95	2.27	130	90
Oct. 1 A.M.†	18.87	14.45	131	91	2.58	1.78	145	100
1 P.M.	17.35	12.09	144	100	2.11	1.53	138	95
2 A.M.	17.10	11.70	146	101				

* See table I.
† Spray removed from both surfaces.

As in experiment III, no visible injury was noted throughout this experiment, yet both photosynthesis and transpiration were reduced by Bordeaux 4–6–100 applied at 82° F.

Experiment V (temp. 100° F.)

The temperature in this experiment is comparable with mid-day temperatures often occurring on hot days in July or August in the field.

The data given in table V and figure 2–E represent only the average of

TABLE V

THE INFLUENCE OF BORDEAUX MIXTURE 4–6–100 ON THE RATE OF PHOTOSYNTHESIS AND TRANSPIRATION OF STAYMAN APPLE LEAVES
TEMPERATURE—100° F.
AVERAGE FOOT CANDLES ON CHECK LEAVES—1300
AVERAGE FOOT CANDLES ON TEST LEAVES—3667
AVERAGE VAPOR PRESSURE—3.63 MM. OF Hg

Date 1940	Apparent photosynthesis				Transpiration			
	CO_2/100 cm.²/hour		Expected rate $\frac{A \times 100}{B}$	Percentage of expected rate $\frac{C \times 100}{182^*}$	H_2O/100 cm.²/hour		Expected rate $\frac{E \times 100}{F}$	Percentage of expected rate $\frac{G \times 100}{168^*}$
	Test	Check			Test	Check		
	A	B	C	D	E	F	G	H
	mg.	*mg.*		%	*gm.*	*gm.*		%
Oct. 4	29.05	16.39	177	97	5.61	3.61	155	92
5	21.87	10.50	208	114	4.80	2.64	182	108
6	21.81	13.12	166	91	7.90	4.71	168	100
7	22.43	12.74	176	97				
Average			182*	100			168*	100
8	19.70	13.58	145	80	4.78	3.18	150	89
1st 9	16.57	11.33	146	80	4.29	2.83	152	90
spray 10	20.32	14.93	137	75	4.84	3.07	158	94
11	24.82	17.80	139	76	4.61	2.47	187	111
12	21.30	13.93	153	84	3.92	2.56	153	91
13	22.03	14.67	150	82				
2nd 14	24.24	16.54	147	81	5.86	3.18	184	110
spray 15	20.10	13.30	151	83	3.62	2.31	153	91
16								
17	19.74	15.12	131	72	4.26	2.73	156	93
18	25.10	15.49	162	89	5.32	4.09	130	77
3rd 19	22.73	15.85	143	79				
spray 20	24.01	14.91	161	88	4.95	3.01	164	98
21	20.11	14.89	135	74	4.31	2.64	163	97
22†	18.94	9.90	213	117	4.52	2.76	164	98
23	38.24	19.02	201	110	4.12	2.38	173	103
24‡	30.59	17.49	175	96	4.13	2.08	199	118
25								

* See table I.
† Spray removed from the upper surface.
‡ Spray removed from the lower surface.

five check as compared with the usual six test leaves, owing to the loss of a check leaf during the experiment. The Stayman Winesap trees used in this test were similar to those described in the previous experiment. After the pre-spray relationship had been established over four days, three applications of Bordeaux 4–6–100 were made at four- and six-day intervals. As previously mentioned, the spray treatments were made in the early evening after the bank of lights in the chamber had been shut off. Only a single 200-watt light was used during spray application.

In contrast to the four experiments heretofore discussed the rate of photosynthesis was reduced rather markedly—20 per cent. immediately following the first spray. In previous experiments it may be remembered that the decrease in photosynthesis was small or questionable in some cases after the first spray; a definite reduction was usually evident, however, after the second or third spray application. The curve representing percentage of expected rate of carbon dioxide absorption (fig. 2–E) levels off somewhat after the initial drop in photosynthesis following the first spray treatment, and was practically unchanged even after the second and third treatment with Bordeaux. The rate of transpiration was not significantly influenced except on October 18, the morning after the third spray application, when the rate of transpiration dropped 23 per cent. Photosynthesis had fully recovered two days later, however. If the experiment as a whole is considered, it is apparent that transpiration was below its expected rate in nine out of eleven individual determinations after spray was applied to the test leaves.

The removal of the spray residue from the upper surface of the test leaves the evening of October 21 brought about immediate recovery in photosynthesis the following morning. If anything, the rate of transpiration appeared to recover after the spray was removed from both surfaces of the leaves. It should be mentioned that there were no signs of visible injury either before or after the removal of the spray deposit.

From this experiment and those conducted at 70° and 82° F. it seems evident that applications of Bordeaux 4–6–100 will reduce the rate of photosynthesis and transpiration of apple leaves even in the absence of visible injury. The fact that photoysnthesis fully recovered after the removal of the spray residue from the upper surface only, indicates that it is largely through the upper leaf surface that Bordeaux mixture causes a reduction in the rate of carbon dioxide absorption.

Experiment VI (temp. 75° F.)

It is often the custom to use a 2–6–100 Bordeaux in the orchard spray program. Hence, the effect of this more dilute mixture of Bordeaux on leaf metabolism was studied.

A single Stayman Winesap apple tree was selected for this investigation since there were present a sufficient number of desirable leaves on the one vigorously growing shoot. Data given in table VI and figure 2–F represent

TABLE VI

THE INFLUENCE OF BORDEAUX MIXTURE 2–6–100 ON THE RATE OF PHOTOSYNTHESIS AND TRANSPIRATION OF STAYMAN APPLE LEAVES

TEMPERATURE—75° F.
AVERAGE FOOT CANDLES ON CHECK LEAVES—1050
AVERAGE FOOT CANDLES ON TEST LEAVES—812
AVERAGE VAPOR PRESSURE—10.97 MM. OF Hg

DATE 1940		APPARENT PHOTOSYNTHESIS				TRANSPIRATION			
		CO_2/100 CM.²/ HOUR		EXPECTED RATE $\frac{A \times 100}{B}$	PERCENTAGE OF EXPECTED RATE $\frac{C \times 100}{112^*}$	H_2O/100 CM.²/ HOUR		EXPECTED RATE $\frac{E \times 100}{F}$	PERCENTAGE OF EXPECTED RATE $\frac{G \times 100}{104^*}$
		TEST	CHECK			TEST	CHECK		
		A	B	C	D	E	F	G	H
		mg.	*mg.*		%	*gm.*	*gm.*		%
Feb.	19	19.70	16.85	117	104	2.44	2.42	101	97
	20	20.48	18.70	110	98	1.29	1.35	96	92
	21	19.50	18.32	106	95	1.70	1.56	109	105
	22	16.33	14.50	113	101	1.50	1.35	111	107
Average				112*	100			104*	100
1st spray	23	13.56	13.95	97	87	1.60	1.70	94	90
	24	17.53	17.21	102	91	1.52	1.42	107	103
	25	17.49	17.80	98	88	1.44	1.52	95	91
2nd spray	26	19.98	20.44	98	88	1.40	1.45	97	93
	27	15.27	15.45	99	88	1.72	1.81	95	91
	28	14.85	15.26	97	87	1.89	1.90	99	95
	29	13.94	15.74	89	79	1.34	1.56	86	83
Mar.	1					1.30	1.29	101	97
3rd spray	2	10.12	11.12	91	81	1.51	1.57	96	92
	3	10.94	12.27	89	79	1.62	1.63	99	95
	4†	12.39	12.19	102	91	1.70	1.65	103	99
	5	15.27	14.69	104	93	1.73	1.66	104	100

* See table I.
† Spray removed from both surfaces.

the average of five check leaves and six test leaves. A relationship was established between the rates of photosynthesis and transpiration of the test and check leaves from February 19 to 22. Applications of Bordeaux 2–6–100 were made on the evenings of February 22, 25, and 29. It is apparent from table VI and the graph in figure 2–F that a gradual reduction in photosynthesis occurred following each application of spray. On February 29, after three applications of Bordeaux 2–6–100, photosynthesis was reduced 21 per cent. The transpiration rate was affected in a similar manner

but to a somewhat lesser degree. Here again, as in all experiments conducted at 70° F. and above, the removal of the spray residue brought about a definite recovery in photosynthesis. Two days after spray removal the rate of carbon dioxide absorption was but 6 per cent. lower than the expected rate. Transpiration also recovered following spray removal but as shown in figure 2–F the rate of transpiration had begun to recover on March 1, three days prior to spray removal. That photosynthesis tends to recover in 10 days to two weeks after a single application of Bordeaux is shown later in experiment X.

The data presented in this experiment indicate a reduction in photosynthesis and transpiration of apple leaves treated three times at 100° F. and at relatively short intervals with Bordeaux 2–6–100. This decrease,

TABLE VII

THE INFLUENCE OF BORDEAUX MIXTURE 4–6–100 ON THE RATE OF PHOTOSYNTHESIS AND TRANSPIRATION OF STAYMAN APPLE LEAVES GROWING UNDER LOW WATER CONDITIONS

TEMPERATURE—85° F.
AVERAGE FOOT CANDLES ON CHECK LEAVES—1352
AVERAGE FOOT CANDLES ON TEST LEAVES—1708
AVERAGE VAPOR PRESSURE—15.11 MM. OF Hg

	DATE 1940	APPARENT PHOTOSYNTHESIS				TRANSPIRATION			
		CO_2/100 CM.²/ HOUR		EXPECTED RATE $\frac{A \times 100}{B}$	PERCENTAGE OF EXPECTED RATE $\frac{C \times 100}{94^*}$	H_2O/100 CM.²/ HOUR		EXPECTED RATE $\frac{E \times 100}{F}$	PERCENTAGE OF EXPECTED RATE $\frac{G \times 100}{105^*}$
		TEST	CHECK			TEST	CHECK		
		A	B	C	D	E	F	G	H
		mg.	mg.		%	gm.	gm.		%
	June 20	22.27	23.88	93	99	3.08	3.16	97	91
	21	19.52	19.98	98	104	3.29	2.99	110	105
	22	21.80	23.44	93	101	3.77	3.58	105	100
	23								
	24	26.28	26.61	99	105	3.42	3.21	107	102
	25	18.48	21.43	86	92	2.59	2.46	105	100
Average				94*	100			105*	100
1st spray	26	7.43	9.80	76	81	1.92	1.64	117	111
	27	7.55	10.38	73	78	1.14	1.07	107	102
	28					1.33	1.21	110	105
	29	10.71	14.51	74	79	2.35	2.32	101	96
2nd spray	30	4.56	7.18	64	68	2.05	2.16	95	90
	July 1	8.66	10.94	79	84	2.00	2.12	94	90
	2	10.61	13.10	81	86				
3rd spray	3	4.74	7.44	64	68	1.30	1.26	103	98
	4	10.27	17.24	60	64	1.96	1.85	94	90
	5	3.25	5.40	60	64	1.74	1.82	96	91

* See table I.

however, was not markedly different from that observed following a similar number of sprays of Bordeaux 4–6–100 to apple foliage maintained at temperature of 70° F.

Experiment VII (temp. 85° F.)

Several investigators (3, 29) have observed that Bordeaux sprayed plants growing under drought conditions were stunted, wilted, or suffered

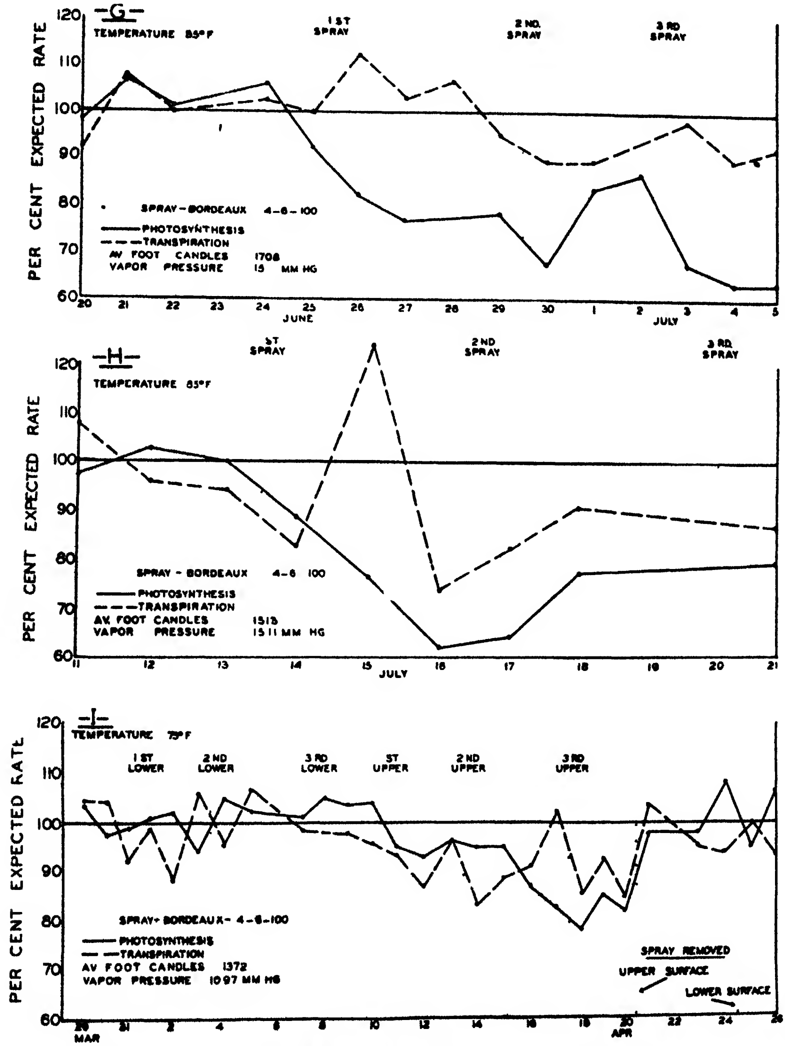

Fig. 3. (G) and (H). The effect of Bordeaux mixture 4–6–100 on the rate of photosynthesis and transpiration of apple leaves growing under drought conditions. (I). The effect of Bordeaux mixture 4–6–100 on the rate of photosynthesis and transpiration of apple leaves when sprayed separately on the upper and lower surfaces.

other deleterious effects not noted on similar unsprayed plants growing under identical conditions. It is generally assumed that such growth responses instigated by Bordeaux were correlated directly with an increased rate of transpiration. The results obtained in this experiment show how a young apple tree growing under low water conditions responded in leaf activity when sprayed with Bordeaux. A single Stayman Winesap tree bearing two vigorous shoots was employed in this investigation. Before any spray was applied the soil was allowed to dry until the leaves showed signs of wilting. Every evening sufficient water was added to the soil so that the leaves regained turgidity and remained so at least for the duration of the test the following morning.

When a relationship had been established between the check and the test leaves a spray of Bordeaux 4–6–100 was applied, as in all previous experiments, to the test leaves on June 25, June 29, and July 2. It may be seen in table VII and figure 3–G that the rate of carbon dioxide absorption was immediately reduced 20 per cent. by the first application of spray. After this initial reduction in photosynthesis, the level of carbon dioxide absorption remained approximately the same until the morning following the second spray when a 32 per cent. reduction occurred. The rate of carbon dioxide absorption partially recovered on July 1 but dropped again the next day and remained about 25 per cent. below the expected rate after the third spray.

In contrast to all previous experiments, the rate of transpiration rose slightly the day following the first application of Bordeaux. This slight increase was only temporary, however, for there was no other significant change in the rate until after the second spray treatment when a 10 per cent. decrease was measured. This minor reduction in water-vapor loss persisted for the remainder of the experiment. At no time during this experiment did the test leaves appear to wilt before the neighboring unsprayed leaves on the same shoot, or *vice versa.*

Experiment VIII (temp. 85° F.)

To check the validity of the results obtained in experiment VII the entire investigation was duplicated, as nearly as possible, in this experiment. As in experiment VII this Stayman Winesap tree possessed two vigorously growing shoots, on which the test leaves were alternately selected for the study.

The data given in table VIII and figure 3–H represent the average values of photosynthesis and transpiration for six test and six check leaves during 2½-hour daily determinations. It is evident from figure 3–H that photosynthesis was reduced markedly by a single application of Bordeaux 4–6–100 under drought conditions. In fact, by July 16, three days after

TABLE VIII

THE INFLUENCE OF BORDEAUX MIXTURE 4–6–100 ON THE RATE OF PHOTOSYNTHESIS AND TRANSPIRATION OF STAYMAN APPLE LEAVES GROWING UNDER LOW WATER CONDITIONS
TEMPERATURE—85° F.
AVERAGE FOOT CANDLES ON CHECK LEAVES—1640
AVERAGE FOOT CANDLES ON TEST LEAVES—1513
AVERAGE VAPOR PRESSURE—15.11 MM. OF Hg

Date 1940		Apparent photosynthesis				Transpiration			
		CO_2/100 cm.^{2}/hour		Expected rate $\frac{A \times 100}{B}$	Percentage of expected rate $\frac{C \times 100}{100^*}$	H_2O/100 cm.^{2}/hour		Expected rate $\frac{E \times 100}{F}$	Percentage of expected rate $\frac{G \times 100}{108^*}$
		Test	Check			Test	Check		
		A	B	C	D	E	F	G	H
		mg.	mg.		%	gm.	gm.		%
July	11	6.67	6.84	98	98	1.29	1.11	116	107
	12	13.56	13.34	102	102	1.24	1.18	105	97
	13	12.06	12.07	100	100	1.19	1.15	103	95
Average				100*	100			108*	100
1st spray	14	7.64	8.45	90	90	0.83	0.93	89	83
	15	6.57	8.37	78	78	0.88	0.65	135	125
	16	3.21	5.20	62	62	0.69	0.84	82	76
2nd spray	17	4.02	6.38	63	63	0.71	0.80	89	82
	18	9.11	11.66	78	78	1.61	1.63	99	92
	19								
3rd spray	20								
	21	5.86	7.64	79	79	1.87	2.05	91	84

* See table I.

treatment, a 38 per cent. reduction in carbon dioxide absorption ensued. Although photosynthesis partially recovered two days after the second spray, as it did in experiment VII, photosynthesis still remained 20 per cent. below the expected rate for the duration of this experiment.

In experiment VII, a slight increase in transpiration was noted the first day following treatment with Bordeaux 4–6–100. In contrast to those results the rate of water-vapor loss was reduced the day after an application of spray in this investigation, but rose 25 per cent. above the expected rate the second day after treatment. This marked rise was only temporary, however, for the transpiration rate fell below the expected rate on the next day, July 16, where it remained after two additional Bordeaux sprays. It should be remembered that since the absolute rates of transpiration were extremely low in this experiment, often less than 1 gm. per 100 sq. cm. per hour, slight changes in the transpiration rate seem great when expressed on a percentage basis.

A comparison of the graphs for experiments VII and VIII in figure

3–G and 3–H shows that although the curves for the two experiments are not identical the trends of both photosynthesis and transpiration are similar. The results of these two investigations indicate that probably several of the deleterious effects of Bordeaux mixture on plants growing under drought conditions (**28**) are not entirely due to a greatly increased transpiration rate but, at least in part, to a reduced rate of photosynthesis.

Experiment IX (temp. 75° F.)

In experiments III, IV, and VI both photosynthesis and transpiration completely recovered when the spray deposit was removed from both leaf surfaces. The results of experiment V indicate, however, that the spray residue on the upper surface was largely responsible for reduction in the rates of these two processes following applications of Bordeaux mixture to both leaf surfaces.

This experiment was designed to determine whether the spray material on the upper, lower, or both surfaces was primarily important in inducing the lowered rates of photosynthesis and transpiration observed previously.

The data for this experiment given in table IX and figure 3–I represent the average rates of carbon dioxide absorption and transpiration of six test and six check leaves. The tree selected was a Stayman Winesap bearing two vigorously growing shoots.

After the usual pre-spray relationship was established between the check and the test leaves, Bordeaux 4–6–100 was sprayed on the lower surface of the test leaves on March 31, April 3, and April 7. The rates of both photosynthesis and transpiration were not significantly influenced by these three treatments. Consequently, the upper surface of the test leaves was sprayed with Bordeaux 4–6–100 on April 11, 13, and 17. The rate of carbon dioxide absorption gradually dropped succeeding each application of spray material to the upper surface. By April 18, the day following the third treatment to the upper surface, the photosynthetic rate was 22 per cent. below the expected rate. The transpiration rate, which was unaffected by three sprays of Bordeaux applied to the lower surface, was lowered somewhat by an equal number of sprays to the upper surface.

It was interesting to note that the removal of the spray deposit from the upper surface brought about an immediate recovery in both photosynthesis and transpiration (table IX, fig. 3–I). It is evident from the results of this experiment that the influence of Bordeaux in lowering the rates of photosynthesis and transpiration of apple leaves was brought about largely, if not completely, by spray material residing on the upper leaf surface rather than by spray residue existing on the lower surface.

Experiment X (temp. 70° F.)

Amos (**1**) observed that although the rate of photosynthesis of grape,

TABLE IX

THE INFLUENCE OF BORDEAUX MIXTURE 4–6–100 ON THE RATE OF PHOTOSYNTHESIS AND TRANSPIRATION OF STAYMAN APPLE LEAVES

TEMPERATURE—75° F.
AVERAGE FOOT CANDLES ON CHECK LEAVES—1169
AVERAGE FOOT CANDLES ON TEST LEAVES—1372
AVERAGE VAPOR PRESSURE—10.97 MM. OF Hg

DATE 1940		APPARENT PHOTOSYNTHESIS				TRANSPIRATION			
		CO_2/100 CM.²/ HOUR		EXPECTED RATE $\frac{A \times 100}{B}$	PERCENTAGE OF EXPECTED RATE $\frac{C \times 100}{120^*}$	H_2O/100 CM.²/ HOUR		EXPECTED RATE $\frac{E \times 100}{F}$	PERCENTAGE OF EXPECTED RATE $\frac{G \times 100}{114^*}$
		TEST	CHECK			TEST	CHECK		
		A	B	C	D	E	F	G	H
		mg.	*mg.*		%	*gm.*	*gm.*		%
Mar.	29	24.69	20.03	123	103	2.04	1.71	119	104
	30	19.38	16.36	118	98	1.85	1.55	119	104
	31	19.95	16.74	119	99	1.97	1.87	105	92
Average				120*	100			114*	100
Apr.	1	19.23	15.84	121	101	1.64	1.45	113	99
1st	2	21.18	17.39	122	102	1.58	1.58	100	88
spray	3	14.71	13.00	113	94	1.97	1.64	120	105
lower§	4	21.28	17.08	125	104	2.06	1.89	109	96
2nd	5	22.03	18.00	122	102	2.08	1.72	121	106
spray	6								
lower§	7	16.14	13.29	121	101	2.17	1.92	113	99
3rd	8	16.47	13.03	126	105				
spray	9	20.99	16.85	125	104	1.93	1.73	112	98
lower§	10	21.99	17.64	125	104	1.46	1.33	110	96
1st	11	22.83	19.99	114	95	1.22	1.25	106	93
spray	12	20.53	18.36	112	93	1.51	1.52	99	87
upper§	13	15.95	13.85	115	96	1.72	1.57	110	96
2nd	14	18.96	16.65	114	95	1.56	1.68	93	82
spray	15	14.57	12.74	114	95	1.69	1.66	102	89
upper§	16	18.91	18.16	104	87	1.57	1.51	104	91
	17	13.81	13.94	99	83	1.32	1.14	116	102
3rd	18	15.29	16.25	94	78	1.50	1.54	97	85
spray	19	14.21	13.85	103	86	1.67	1.57	106	93
upper§	20	13.70	14.05	98	82	1.59	1.64	97	85
	21†	24.85	21.02	118	98	2.05	1.77	116	102
	22								
	23	15.97	13.58	118	98	1.95	1.78	110	96
	24	20.56	15.78	130	108	1.81	1.69	107	94
	25‡	20.06	17.53	114	95	1.87	1.64	114	100
	26	20.61	16.13	128	107	1.51	1.42	106	93

* See table I.
† Spray removed from the upper surface.
‡ Spray removed from the lower surface.
§ Either upper or lower surface of the leaf as indicated.

hop, and Jerusalem artichoke was reduced temporarily by a spray of Bordeaux mixture, the carbon dioxide absorption eventually completely recovered. An effort was made in this experiment to determine whether such a recovery followed applications of Bordeaux mixture to apple foliage, if adequate time were allowed between spray applications.

A single Stayman Winesap tree bearing two shoots which had not set terminal buds was employed in this experiment. The data are given in table X and figure 4–J. A pre-spray relationship between the check and test

TABLE X

THE INFLUENCE OF BORDEAUX MIXTURE 4–6–100 ON THE RATE OF PHOTOSYNTHESIS AND TRANSPIRATION OF STAYMAN APPLE LEAVES

TEMPERATURE—70° F.
AVERAGE FOOT CANDLES ON CHECK LEAVES—2268
AVERAGE FOOT CANDLES ON TEST LEAVES—2226
AVERAGE VAPOR PRESSURE—9.47 MM. OF Hg

DATE 1940		APPARENT PHOTOSYNTHESIS				TRANSPIRATION			
		CO_2/100 CM.^{2}/HOUR		EXPECTED RATE $\frac{A \times 100}{B}$	PERCENTAGE OF EXPECTED RATE $\frac{C \times 100}{112^*}$	H_2O/100 CM.^{2}/HOUR		EXPECTED RATE $\frac{E \times 100}{F}$	PERCENTAGE OF EXPECTED RATE $\frac{G \times 100}{109^*}$
		TEST	CHECK			TEST	CHECK		
		A	B	C	D	E	F	G	H
		mg.	mg.		%	gm.	gm.		%
May	18	27.77	25.43	109	97	2.54	2.37	107	98
	19	26.94	24.08	112	100	2.95	2.73	108	99
	20	26.85	23.46	114	102	1.58	1.42	111	102
Average				112*	100			109*	100
	21	26.94	26.50	102	91	2.15	1.89	114	105
	22	22.83	22.75	100	89	2.11	2.15	98	90
1st spray	23								
	24	21.34	20.35	105	94	2.31	2.16	107	98
	25	20.47	20.99	98	88	2.14	2.10	102	93
	29	18.32	16.11	114	102	2.01	1.89	106	97
	30	20.59	18.54	111	99	1.89	1.89	100	92
	31	21.54	22.88	94	84	1.58	1.53	103	94
June	1	19.45	19.60	99	88	1.77	1.79	99	91
	2	19.74	19.98	99	88	2.05	1.93	106	97
2nd spray	5	20.94	21.54	97	87	1.89	1.92	98	90
	6	22.62	21.99	103	92	1.62	1.54	105	96
	15	16.99	16.33	104	93	2.10	2.21	95	87
	16	16.98	14.41	118	105	2.18	1.85	117	107

* See table I.

leaves was obtained in three days, after which a spray of Bordeaux 4–6–100 was applied to both surfaces of the test leaves on May 21. A 10 per cent. reduction in photosynthesis ensued and remained until May 29 when the

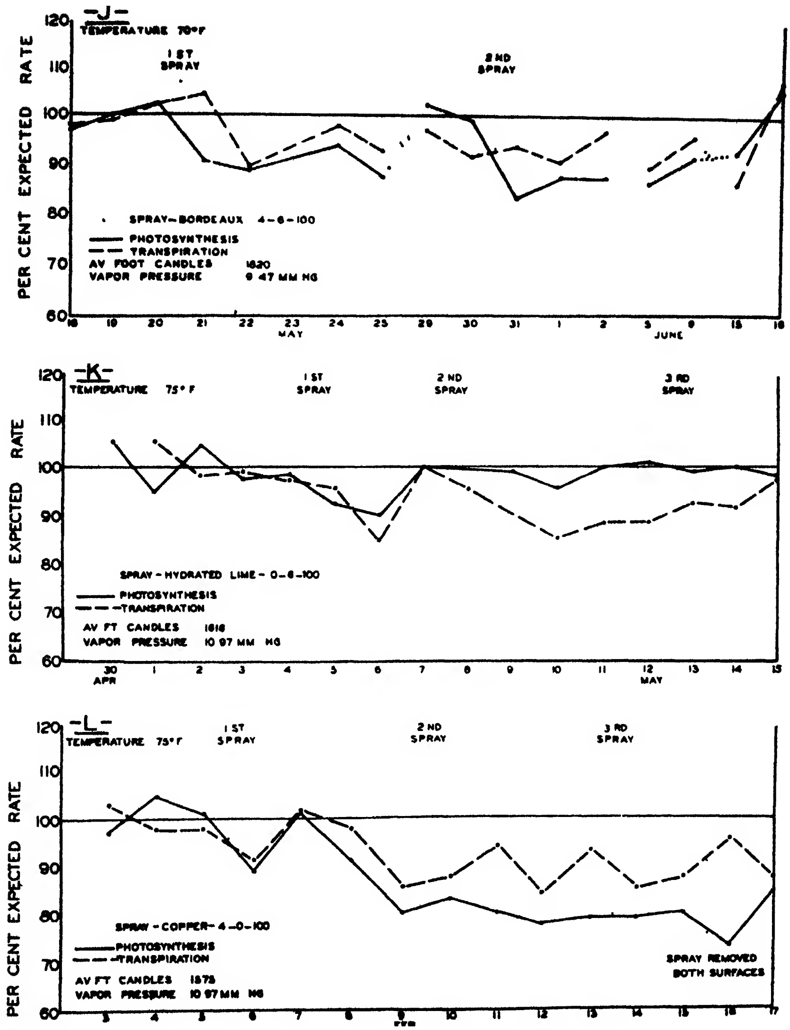

FIG. 4. The effect of (J), Bordeaux mixture 4–6–100, (K), hydrated lime 0–6–100, and (L), copper sulphate 4–0–100, on the rate of photosynthesis and transpiration of apple leaves.

rate of carbon dioxide absorption had completely recovered. On May 30 another application of Bordeaux lowered the rate of photosynthesis 16 per cent. on May 31. The rate of carbon dioxide absorption continued below the expected rate until June 16, which was 17 days after the second application. At this time photosynthesis had again completely recovered. Transpiration was not influenced appreciably by Bordeaux mixture in this experiment although the rate was consistently below the expected rate.

The evidence presented in this experiment and also that obtained by AMOS (1) adds support to the belief that the influence of Bordeaux in reducing the photosynthesis is only temporary and, consequently, more physiological than mechanical.

EXPERIMENT XI (TEMP. 75° F.)

Investigators are not in absolute accord as to whether the lime or the copper sulphate fraction in Bordeaux mixture is responsible for the influence of this spray on both photosynthesis and transpiration. Thus, the effect of a 0–6–100 hydrated lime spray on these two processes was studied in this experiment.

A single Stayman Winesap apple tree bearing two rapidly growing shoots was selected. The data obtained are presented in table XI and figure 4–K.

TABLE XI

THE INFLUENCE OF HYDRATED LIME 0–6–100 ON THE RATE OF PHOTOSYNTHESIS AND TRANSPIRATION OF STAYMAN APPLE LEAVES

TEMPERATURE—75° F.
AVERAGE FOOT CANDLES OF CHECK LEAVES—1617
AVERAGE FOOT CANDLES ON TEST LEAVES—1616
AVERAGE VAPOR PRESSURE—10.97 MM. OF Hg

DATE 1940		APPARENT PHOTOSYNTHESIS				TRANSPIRATION			
		CO_2/100 CM.^{2}/HOUR		EXPECTED RATE $\frac{A \times 100}{B}$	PERCENTAGE OF EXPECTED RATE $\frac{C \times 100}{107^*}$	H_2O/100 CM.^{2}/HOUR		EXPECTED RATE $\frac{E \times 100}{F}$	PERCENTAGE OF EXPECTED RATE $\frac{G \times 100}{105^*}$
		TEST	CHECK			TEST	CHECK		
		A	B	C	D	E	F	G	H
		mg.	mg.		%	gm.	gm.		%
April 30		21.98	19.67	112	105				
May 1		28.12	27.60	102	95	2.28	2.07	110	105
	2	18.66	16.85	111	104	1.90	1.84	103	98
	3	17.55	16.81	104	97	1.79	1.72	104	99
	4	17.25	16.43	105	98	1.98	1.94	102	97
Average				107*	100			105*	100
1st spray	5	22.13	22.09	100	93	2.68	2.66	101	96
	6	16.92	17.60	96	90	1.85	2.09	89	85
	7	23.17	21.57	107	100	1.53	1.46	105	100
2nd spray	8					1.60	1.59	101	96
	9	18.24	17.16	106	99				
	10	21.80	21.22	103	96	1.85	2.09	89	85
	11	24.50	22.81	107	100	1.87	1.82	92	88
	12	23.29	21.56	108	101	2.14	2.33	92	88
3rd spray	13	23.47	22.11	106	99	2.11	2.16	98	93
	14	16.19	15.10	107	100	1.47	1.51	97	92
	15	14.01	13.32	105	98	1.64	1.61	102	97

* See table I.

They represent, as in previous experiments, the average photosynthesis and transpiration of six check and test leaves. After the pre-spray relationship was established the test leaves were sprayed with a 0–6–100 hydrated lime on May 4, 7, and 12.

The curve for photosynthesis presented in figure 4–K shows that sprays of hydrated lime had perhaps a slight effect on this leaf process shortly after the first spray but later there was no significant effect. This effect of hydrated lime may have been due to its alkalinity (**15**). Transpiration, however, was reduced 15 per cent. by May 10, three days after the second spray application. The rate of loss of water-vapor gradually recovered from this low value until May 15 when the rate of transpiration was within 3 per cent. of the expected rate. There was no injury apparent during this experiment.

TABLE XII

THE INFLUENCE OF COPPER SULPHATE 4–0–100 ON THE RATE OF PHOTOSYNTHESIS AND TRANSPIRATION OF STAYMAN APPLE LEAVES

TEMPERATURE—75° F.
AVERAGE FOOT CANDLES ON CHECK LEAVES—1034
AVERAGE FOOT CANDLES ON TEST LEAVES—1575
AVERAGE VAPOR PRESSURE—10.97 MM. OF Hg

DATE 1940		APPARENT PHOTOSYNTHESIS				TRANSPIRATION			
		CO_2/100 CM.²/HOUR		EXPECTED RATE $\frac{A \times 100}{B}$	PERCENTAGE OF EXPECTED RATE $\frac{C \times 100}{150^*}$	H_2O/100 CM.²/HOUR		EXPECTED RATE $\frac{E \times 100}{F}$	PERCENTAGE OF EXPECTED RATE $\frac{G \times 100}{129^*}$
		TEST	CHECK			TEST	CHECK		
		A	B	C	D	E	F	G	H
		mg.	*mg.*		%	*gm.*	*gm.*		%
Feb.	3	20.19	13.87	146	97	1.24	0.93	133	103
	4	14.56	9.30	156	104	1.84	1.45	127	98
	5	13.74	9.31	148	99	1.52	1.20	127	98
Average				150*	100			129*	100
	6	14.16	10.57	134	89	1.84	1.57	117	91
1st	7	12.43	8.23	151	101	0.88	0.67	131	102
spray	8	12.71	9.20	138	92	1.67	1.32	127	98
	9	15.37	12.84	120	80	1.60	1.44	111	86
	10	17.55	14.21	124	83	1.78	1.56	114	88
2nd	11	19.02	15.82	120	80	1.72	1.42	121	94
spray	12	8.72	7.46	117	78	1.64	1.51	109	84
	13	11.15	9.39	119	79	2.03	1.69	120	93
3rd	14	9.32	7.84	119	79	1.73	1.56	111	86
spray	15	18.73	11.41	120	80	2.39	2.12	113	88
	16†	11.95	10.82	110	73	1.63	1.31	124	96
	17	13.09	10.36	126	84	1.79	1.58	113	88

* See table I.
† Spray removed from both surfaces.

Experiment XII (temp. 75° F.)

This investigation was designed to determine how copper sulphate 4–0–100 affects photosynthesis and transpiration of apple leaves. Two of the three Stayman trees employed were growing in a dark clay loam soil, while the third tree was rooted in a sandy loam. All trees had formed terminal buds. According to the usual manner, the six check and six test leaves were evenly distributed over these three trees.

The data as given in table XII and figure 4–H show that by February 9, four days after the first application of a 4–0–100 spray the rate of photosynthesis was reduced 20 per cent. No visible copper injury on the test leaves was evident at this time. Injury appeared, however, after the second copper sulphate spray. On February 11 purple spots similar to those described by Crandall (7) were visible on the upper surface of most of the sprayed leaves. The rate of carbon dioxide absorption by the leaves, however, was not reduced further even after the appearance of this visible injury or by a third spray of copper sulphate.

As figure 4–L also shows, the influence of copper sulphate on transpiration was quite similar to its effect on photosynthesis but the reduction was of a lesser magnitude. On February 15, both surfaces of the leaves were wiped with moist absorbent cotton. No significant change in either photosynthesis or transpiration followed this operation.

The results obtained on the influence of hydrated lime and copper sulphate on photosynthesis indicate that the reduced rate of carbon dioxide absorption following applications of Bordeaux is due primarily, if not entirely, to the presence of copper in the Bordeaux mixture. Transpiration, however, appears to be lowered by both copper sulphate and hydrated lime.

Discussion

From the results presented in this paper it seems apparent that Bordeaux mixture has at least a temporary retarding influence on the rate of photosynthesis of apple leaves. Regardless of the temperature, humidity, light intensity, or soil moisture conditions photosynthesis was eventually more or less reduced by applications of Bordeaux. However, in the absence of visible injury, such as that which occurred on the Bordeaux sprayed leaves at temperatures of 50 and 60° F., the rate of photosynthesis completely recovered whenever the Bordeaux residue was removed from the treated leaves. Hoffman (14) also noticed that when photosynthesis was reduced by applications of Bordeaux mixture to apple foliage, the rate of carbon dioxide absorption recovered to its pre-spray relationship following spray removal. He suggested that the shading influence of this spray material probably was sufficiently great to retard the rate of photosynthesis. In an effort to determine what effect residues of Bordeaux mixture and its component parts have

in reducing the intensity of light falling on a leaf, three sprays of Bordeaux 4–6–100, hydrated lime 0–6–100, and copper sulphate 4–0–100 were applied to separate sheets of cellulose acetate and the intensity of light passing through the residues and sheeting was measured with a Weston photometer. The results are given in table XIII.

TABLE XIII

THE EFFECT OF DEPOSITS OF BORDEAUX MIXTURE AND ITS COMPONENT PARTS ON THE AMOUNT OF LIGHT PASSING THROUGH A SHEET OF CELLULOSE ACETATE PAPER

NUMBER OF SPRAYS	PERCENTAGE REDUCTION IN LIGHT INTENSITY		
	BORDEAUX 4–6–100	HYDRATED LIME 0–6–100	COPPER SULPHATE 4–0–100
	%	%	%
1	14.8	12.3	0.0
2	22.5	17.5	1.3
3	27.5	22.5	1.5

The results obtained from one application of Bordeaux and hydrated lime are quite similar to those of HYRE (**16**) who recorded a reduction of 11.9 per cent. in light intensity after a single spray of Bordeaux 8–8–100, and 11.2 per cent. reduction following a spray of hydrated lime 0–8–100.

HEINICKE and HOFFMAN (**13**) found from their experiments with apple leaves that, under field conditions, 1200 foot candles of light were sufficient for maximum rates of carbon dioxide absorption. If it is assumed that about 1200 foot candles were adequate for maximum rates of photosynthesis in these experiments, and that the effect of Bordeaux in retarding photosynthesis is purely mechanical shading, then a reduction in the light intensity reaching the test leaves of 27.5 per cent., due to three sprays of Bordeaux 4–6–100, should have no measurable influence on the rate of photosynthesis; for example, in experiment V. In this particular experiment the light falling on the test leaves prior to spraying averaged 3667 foot candles, yet the rate of carbon dioxide absorption was reduced considerably by only one application of Bordeaux 4–6–100. The results obtained previously by AMOS (**1**) and those presented here in experiment X show that the effect of Bordeaux in depressing photosynthesis may be only temporary. This was brought out in these experiments, since several days after applications of Bordeaux were made the rate of carbon dioxide absorption recovered even though the spray residue was not removed. If either the light intensity or the quality reaching the treated leaves was reduced or altered by Bordeaux sufficiently to reduce photosynthesis, it would not seem reasonable to expect that the rate of this process would regain its pre-spray status as long as the

Bordeaux spray deposit remained on the test leaves. The data indicate, therefore, that the primary retarding influence of Bordeaux mixture on apple leaves was not caused by mechanical shading of the test leaves by Bordeaux, but by some physiological effect brought about by the chemical mixture.

Since under the conditions of these experiments, hydrated lime did not appear to have a detrimental influence on the rate of photosynthesis while copper sulphate distinctly retarded the process, it would seem logical to assume that the copper fraction within Bordeaux was the main causative factor in reducing photosynthesis. The diffusion of copper into the leaf tissue could occur only if copper were present in a soluble form. Therefore, it might seem that no soluble copper would be present in a 4–6–100 Bordeaux which contains more than enough lime to precipitate all the copper sulphate present. PICKERING and BEDFORD (**24**) observed, however, that even a Bordeaux which contained an excess of lime released soluble copper upon weathering. That copper is known to penetrate the tissues of a leaf in appreciable amounts has been shown by DELONG (**8**). Leafhoppers (*Empoasca fabae*) which were allowed to feed upon Bordeaux-sprayed bean leaves died within 4 to 5 days of a characteristic "Bordeaux poisoning." This likewise occurred when leaf-hoppers were confined to feeding upon unsprayed portions of sprayed leaves. FRANK, KRUGER, and EWERT, as quoted by LUTMAN (**18**), and BUTLER (**3**) all observed that Bordeaux-treated leaves retained starch in their chloroplasts longer than those leaves left untreated. EWERT as quoted by LUTMAN (**18**) showed that the action of diastase was retarded by copper compounds, resulting in the increased accumulation and retention of starch in the chloroplasts. This accumulation he attributed not to any increase in starch manufacture following applications of Bordeaux but rather to a decrease in the rate of starch hydrolysis. In order to check the results of workers just mentioned, applications of Bordeaux 4–6–100, hydrated lime 0–6–100, and copper sulphate 4–0–100 were made at three-day intervals until several leaves on Stayman Winesap trees in the greenhouse had received three treatments of one of the aforementioned sprays. Two days after the third spray had been applied the trees were moved to a dark room. By extracting the chlorophyll from the leaves with alcohol and then testing them for starch with an I_2KI solution according to SACHS's method, it was possible to determine whether the sprayed leaves retained starch longer than similar untreated checks. The results obtained from these leaves sprayed with copper sulphate were fairly striking. Starch was retained in the treated leaves long after starch had escaped from the checks. Bordeaux-sprayed leaves in a few cases appeared to possess starch after the checks were free of starch but in no instance were the results as marked as those found in copper sulphate treated leaves. The leaves treated with hydrated lime did not

appear to retain starch any longer than the checks, although BUTLER (**3**) states that potato leaves treated with milk of lime retained starch somewhat longer than the untreated checks. Since the concentration of soluble copper in a Bordeaux spray would be only a small fraction of that in a copper sulphate 4–0–100 spray, it would seem logical to expect that more copper would penetrate the leaf tissues immediately following a spray of copper sulphate than one of Bordeaux. If the degree of diastase inactivation is directly related to the concentration of soluble copper present in the leaf, starch would probably accumulate and be retained to a greater degree in those leaves treated with copper sulphate as compared with those sprayed with Bordeaux. MILLER (**23**) states that it is generally considered that an accumulation of starch within the chloroplasts impairs their activity by interfering with the photosynthetic process which some believe occurs at the surface of the plastids. The reduction in photosynthesis following applications of Bordeaux mixture, therefore, may be directly related to a greater accumulation of starch in the treated leaves. The results of experiment X, however, indicate that the influence of Bordeaux in lowering the rate of photosynthesis may be only temporary since the rate of carbon dioxide absorption regained its pre-spray status about two weeks after spray treatment even though the spray residue remained untouched on the leaves. If the inactivation of diastase, a consequent increase in starch accumulation, and a reduced rate of photosynthesis result from the presence of soluble copper in the leaf tissues, it would not seem reasonable to expect the rate of carbon dioxide absorption to recover until the concentration of soluble copper was either reduced or converted to a form that no longer had an inactivating effect on diastase. MADER and BLODGETT (**19**) found that potato plants sprayed with Bordeaux possessed tubers which contained from 2.5 to 5.5 p.p.m. of copper while unsprayed checks contained only 2.0 to 2.5 p.p.m. of copper. This fact clearly shows that copper from Bordeaux not only penetrates leaf tissues but remains at least partially mobile therein. MAQUENNE and DEMOUSSY as quoted by WLLIS (**27**) state that calcium favors the diffusion of copper into aerial portions of plants and probably also prevents, within limits, its undue accumulation. Since the amount of soluble copper in Bordeaux would, in all probability, be greatest immediately after spraying, a sufficiently high concentration of copper might diffuse into, and remain within, the leaf tissues at this time to retard diastase activity, cause starch to accumulate, and depress the rate of photosynthesis. The amount of soluble copper diffusing into the leaf would gradually decrease as the concentration of soluble copper in the Bordeaux on the leaves decreased.[5] Since the copper penetrating the leaf tissues appears to remain at least partially mobile therein, the rate of movement of soluble copper out of the leaf tissue might eventually exceed the inward movement of copper from the Bordeaux residue upon the leaf

surface. When the concentration of internal copper was no longer sufficiently great to inactivate diastase, the rate of photosynthesis would theoretically recover to its pre-spray status even though the Bordeaux spray deposit remained intact on the leaves. If the spray residue were removed within 3 to 5 days after spraying and no visible injury resulted, as in the experiments performed at 70° F. and above, the recovery in photosynthesis which followed such treatment might have been due to the removal of the diffusible copper remaining in the spray sediment on the leaf.

In the mass of literature concerned with the effects of Bordeaux on transpiration, a number of theories have been proposed as explanations of the mechanism involved. Since the results obtained by the investigators are not in complete accord, it is not surprising that their theories are somewhat conflicting. LEBANDER as quoted by MILLER (**23**) suggests that certain light rays are excluded by the Bordeaux residue on the sprayed leaves, thus reducing transpiration. MARTIN and CLARK (**20**) suggest that absence of increase in transpiration of potato leaves after a second application of Bordeaux mixture was due to the clogging of the stomata by the spray particles. This theory has been partially substantiated by the later work of KRAUSCHE and GILBERT (**17**) who definitely show that increase in transpiration following applications of Bordeaux are not due to increases in stomatal transpiration. They found, in fact, that applications of Bordeaux to the lower surface of tomato leaves, where the stomata are most numerous, resulted in a reduced rate of transpiration during the day. It was thought by BUTLER (**3**) that Bordeax and milk of lime were opaque to radiations of long wave lengths, "therefore, under conditions favorable for radiation, sprayed plants cool less rapidly than non-sprayed plants and thus transpire more freely." This assumption does not appear sound since TILFORD and MAY (**26**) comparing the temperature of Bordeaux sprayed potato leaflets with untreated leaflets in the sun, found by means of thermocouple measurements, that leaflets sprayed with a 4–4–50 Bordeaux averaged 1.45° C. lower in temperature than the unsprayed leaflets. They also observed that the whiter the spray, the greater the cooling effect. WILSON and RUNNELS (**29**) suggest that "the accelerating influence of a Bordeaux film on the transpiration rate is due chiefly to a change in the rate of cuticular transpiration." HORSFALL and HARRISON (**15**) have combined the ideas as presented by MARTIN and CLARK (**18**), KRAUSCHE and GILBERT (**17**) and WILSON and RUNNELS (**29**) by stating that "Bordeaux mixture has a two-way action on transpiration—it accelerates cuticular transpiration and curtails stomatal transpiration." They also believe that "the alkalinity of Bordeaux saponifies the cuticle and decreases its resistance to water loss."

Under the conditions of these experiments both stomatal and cuticular transpiration of mature apple leaves were measured simultaneoulsy. Ex-

cept for a single determination in each of the experiments concerned with Bordeaux's influence on transpiration under drought conditions, Bordeaux mixture and its component parts appeared to have either no effect or a retarding influence on the rate of transpiration in all experiments. If it is assumed that Bordeaux mixture has a retarding influence on stomatal transpiration and an accelerating effect on cuticular transpiration (**15**), the lowered rates of foliar transpiration observed in these experiments might be due in part to the depressing effect of Bordeaux on stomatal transpiration. The rate of loss of water vapor from the cuticle in most species of plants in the temperate zone is less than 10 per cent. of the total foliar transpiration (**22**). It appears, therefore, that even though cuticular transpiration may have been somewhat accelerated by Bordeaux, the effect was not of sufficient magnitude to counterbalance the depressing influence of this spray on stomatal transpiration. Most other investigators who have proposed that Bordeaux mixture increases the rate of cuticular transpiration have conducted their research on relatively young herbaceous plants such as Coleus, potato, and tomato which have a relatively thin cuticle as compared with that on mature apple foliage. Consequently, the corroding effect of Bordeaux on mature apple leaves may not be sufficient, in most cases, to greatly alter the rate of cuticular transpiration.

Although the stomata may be partially "clogged" by the spray residue, and thus tend to reduce stomatal transpiration, the results of TILFORD and MAY (**26**) offer another explanation for a general reduction in transpiration due to a Bordeaux spray. If, in the experiments reported here the temperature of Bordeaux-sprayed leaves was lowered from 1 to 2° C., as they state in case of sprayed potato leaflets, a significant change in the vapor pressure gradient between the leaf and the external air would have taken place. CURTIS (**6**) shows that a 1 to 2° C. reduction in leaf temperature, between a range of 10 and 40° C., is equivalent to raising the external humidity as much as 5 to 14 per cent. Since the rate of transpiration is governed by the steepness of the vapor pressure gradient existing between the intercellular spaces and the external atmosphere, any comparative decrease in vapor pressure within the leaf, other conditions remaining constant, would reduce this gradient and lower the rate of transpiration. Under the conditions of some of the experiments presented here this mechanism might have been sufficiently important to be responsible, in part, for significant reductions in the rate of transpiration of leaves sprayed with Bordeaux and hydrated lime.

On the basis of the results in this paper which show a reduction in transpiration of apple leaves due to Bordeaux Mixture, it is difficult to explain an observation made by ANDERSON (**2**) who noted in Illinois during a dry summer period that apple trees sprayed with Bordeaux mixture wilted

soon after the spray application while nearby unsprayed trees showed no signs of wilting. Trees sprayed with Bordeaux plus oil, however, appeared similar to the unsprayed trees. Although it would seem that the oil counteracted an increase in transpiration due to the presence of Bordeaux alone on the leaves (**28**), this may not be the sole or correct answer. It has been shown by the results obtained here and elsewhere (**3, 18**) that the presence of soluble copper on the leaf results in its penetration and partial hindrance to the change of starch to sugar and translocation of elaborated materials out of the leaf. If this should be the case, leaves sprayed with Bordeaux probably would have a lower diffusion pressure deficit between the mesophyll cells of the leaves and the soil solution, as compared with unsprayed leaves of the check trees. Hence, sprayed leaves may wilt because of their inability to obtain as much water as the unsprayed leaves, even though their transpiration rates were approximately the same as those of the checks. The presence of oil in the spray mixture may reduce the amount of soluble copper entering the leaf and thus its resulting effect on diastase activity.

In this connection it might be mentioned also that the wilting of Bordeaux sprayed trees growing under drought conditions may be due to the more rapid entrance of soluble copper into the leaf when the leaves are in a more or less flaccid condition. The toxic action of the copper, when the leaves are in a somewhat wilted condition, may bring about a partial loss of semi-permeability of the cell membranes of the sprayed leaves. When the leaves are in a turgid condition, it is assumed that this would not be the case and under such conditions there actually would be a reduction in transpiration as shown in this paper. That this may be the case is suggested in the results of experiments VII and VIII where apple leaves showed in both experiments a slight increase in transpiration a day or two after the Bordeaux had been applied to young apple trees growing under low soil moisture conditions. Whether this temporary, slight increase in transpiration is important in the total effect may be doubted; but at least it suggests another angle to the problem which should be investigated further. There appears to be a concensus of opinion among fruit growers in Ohio, Indiana, and Illinois that the presence of Bordeaux on apple leaves during a dry period in summer results in increased yellowing and dropping of foliage. It would seem from the results presented here that this yellowing and dropping of leaves could not be explained entirely, or perhaps at all, by a greatly increased transpiration by the Bordeaux-treated trees.

Summary

1. The influence of Bordeaux mixture and its component parts on the rate of photosynthesis and transpiration of Stayman Winesap apple leaves

was measured in an environment-control chamber where temperature, light, and humidity were controlled.

2. Under the conditions of these experiments photosynthesis was reduced at least temporarily by applications of Bordeaux mixture regardless of the temperature, light, humidity, or soil moisture conditions.

3. The data presented indicate that the influence of Bordeaux mixture on the photosynthetic process is primarily physiological rather than mechanical. The soluble copper fraction within Bordeaux appears to be directly related to the retarding effect of this spray on photosynthesis.

4. The results of these experiments indicate that the temperature at which Bordeaux may be expected to instigate visible injury to apple foliage lies between 60° and 70° F.

5. The transpiration rate of illuminated mature apple leaves appears to be uninfluenced or somewhat depressed by Bordeaux mixture and its component parts.

6. The general effect of Bordeaux mixture applied to young apple trees growing under low soil moisture conditions was a slightly reduced rate of transpiration.

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FORMIC ACID FORMATION IN ALCOHOLIC FERMENTATION

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(WITH ONE FIGURE)

Introduction

The possible occurrence, origin, and rôle of formic acid in alcoholic fermentation has been a particularly controversial subject. KRUIS and RAYMAN (**26**) observed formic acid in fermented liquids and attributed it to a direct action of air upon some constituent of the wort. The majority of the investigators who have observed the presence of this acid in fermented liquids, however, believed it to play some rôle in the fermentations of sugar by yeast. Among these were LIEBERMANN (**27**), KITICSAN (**23**), DUCLAUX (**5**), KHOUDABACHIAN (**22**), THOMAS (**37**), FRANZEN and STEPPUHN (**16, 17**), KOSTYCHEV (**25**), ESAU (**9**), and JOSLYN and DUNN (**20**). Formic acid or formate utilization by yeasts has also been reported by DUCLAUX (**5**), THOMAS (**37**), FRANZEN and STEPPUHN (**16, 17**), FRANZEN (**14**), and NEUBERG and TIR (**30**). On the other hand, PAKES and JOLLYMAN (**33**) failed to detect any formate utilization by yeasts. Formate utilization by bacteria has been well established by OMELIANSKY (**31**), FRANZEN *et al.* (**12, 13, 15**), GREY (**18**), and PAKES and JOLLYMAN (**33**).

DUCLAUX (**5**) suggested that yeasts produced formic acid when grown under unfavorable environmental conditions. Formic acid was considered as an intermediate in alcoholic fermentation according to a theory developed by WOHL (**44**) and extended by SCHADE (**34**). EHRLICH (**7, 8**) favored the WOHL-SCHADE hypothesis, although he suggested that formic acid could be obtained from amino acids, ammonia, and also an aldehyde of the next lower acid in the particular series being formed. LÖB (**8**) objected to SCHADE's hypothesis on the basis that the latter's reactions occur under entirely different chemical conditions than are required for alcoholic fermentation. FRANZEN and STEPPUHN (**16**) reported that there was initially a slight accumulation of formic acid and subsequently a decrease in media containing formates. This, however, does not constitute evidence, as they believed, for its rôle as intermediate in alcoholic fermentation (**10**). NEUBERG and KERB (**29**) testing the SCHADE hypothesis, failed to obtain alcohol from mixtures of formates, acetaldehyde, and yeast. KOSTYCHEV (**25**) did not regard formic acid as a fermentation product, but rather as a product of endogenous metabolism of the cell. THOMAS (**37**) inclined toward the view that the production of formic acid is intimately bound up with the breakdown of sugar but OPPENHEIMER (**32**) did not believe that formic acid played any rôle in normal alcoholic fermentation.

WIELAND and SONDERHOFF (**40**) have indicated recently that formic acid occurs among the products of aerobic and anaerobic decomposition of citric acid by impoverished yeast, but bacterial contamination was found. In recent years, formic acid has not been generally regarded as an intermediate product of alcoholic fermentation; its occurrence, origin, and rôle still remain unknown.

A study of formic acid formation and utilization by certain yeasts was made and it was found that insignificant quantities of formic acid are formed when particular care is taken to avoid bacterial contamination of fermentation mixtures. Some evidence for utilization of formic acid is presented.

Methods

In addition to the two yeasts, 66 and Burgundy, used in an earlier investigation in this laboratory by JOSLYN and DUNN (**20**), five other closely related strains of yeast were used. They were all found, when classified according to the methods of STELLING-DEKKER (**36**), to be strains of *Saccharomyces cereviseae* (HANSEN) DEKKER. The Champagne strain, as well as Burgundy, was originally from the collection of PACOTTET of France (**2**). Numbers 2338 and 2368 were obtained from the American Type Culture Collection in July, 1937. The former was labelled *Saccharomyces ellipsoideus* and the latter, *Saccharomyces cereviseae*. The Tokay strain of this laboratory was originally from California Fruit Industries, Ltd. Yeast number 66 was isolated by CRUESS (**4**) and the Frei Brothers' (FB) strain was isolated by the writers from a naturally fermenting vat of wine at Frei Brothers' Winery, Healdsburg, California, 1935 vintage.

The media used for the experiments included both natural media, grape juice and wort, and an artificial medium which was a modification of Williams and Saunder's medium (**41**) with the rare elements and asparagine replaced by a small quantity of yeast extract. This artificial medium contained 200 grams dextrose, 3 grams ammonium sulphate, 2 grams potassium acid phosphate, 0.25 grams $CaCl_2 \cdot 2H_2O$, 0.25 grams $MgSO_4$, and 30 ml. yeast extract per liter; the yeast extract contained 0.25 per cent. nitrogen, and 1.5 per cent. total solids.

The media were bottled in cotton stoppered bottles of suitable size for each respective experiment and sterilized in flowing steam for a period of one hour on each of three successive days.

Yeast starters were generally prepared by transferring a loopful of yeast from a young stock agar slant to a small volume of the particular medium to be used in the experiment. This was transferred after two or three days to another portion of the same medium. After two more days the experimental media were inoculated, using serological pipettes, with a volume of

this second culture, amounting to one per cent. of the volume of the medium being inoculated. The fermentations were conducted at room temperature, 18 to 25° C. (average 22°).

The total volatile acidity was determined in duplicate by titrating in the hot 100 ml. of steam distillate from a 10-ml. sample. Formic acid was determined by the total extraction mercury reduction method of VON FELLENBERG (**11**) using 50-ml. samples. In addition, the DYER (**16**) distillation procedure for formic acid determination was used on a few of the samples. pH was determined by the quinhydrone electrode. Sugar was determined by HASSID's (**19**) modification of WHITMOYER's procedure.

Most of the fermentations were made in duplicate. There was close agreement between duplicates and only the averages are included in the tables.

Data and discussion

FORMIC ACID FORMATION DURING FERMENTATION OF GRAPE JUICE

Tall eight-liter bottles fitted with sampling tubes, were filled with six liters of Thompson Seedless grape juice having 116 milli-mols total reducing sugar (as dextrose), 0.12 milli-equivalents total volatile acid, and 0.08 milli-equivalents formic acid per 100 ml.; the pH was 3.67. Samples of approximately 100 ml. were withdrawn at intervals, determined by the relative rate of fermentation, over a total period of 75 days (table I). The yeasts were removed from the samples by centrifuging, and they were then frozen and stored at − 18° C. until analyses could be made. When the last sample had been withdrawn, the yeast sediment was examined microscopically. In addition dilution plates and streak plates were made on suitable media to verify the purity of the cultures. No contaminants were found.

The data obtained for two of the yeasts, 66 and Champagne, are summarized in table I. The rapid rise in total volatile acidity during the fermentation of the first half of the sugar is in agreement with the results reported in a previous paper (**20**). Individual differences as to total amount and relative rate of formation occurred among the seven strains, but the general trend was similar for all the strains. A study of the formic acid data reveals that, except possibly in the initial stages of fermentation, there was little, if any, accumulation of formic acid. In order to determine whether the substance from the initial medium which caused the precipitation of calomel was actually formic acid, an ether extract was tested with cerous nitrate (**1, 24**) and characteristic crystals were formed, indicating that formic acid was present. The quantities of formic acid present in the medium at the various stages fluctuated somewhat irregularly, especially at the beginning of fermentation. This may have been due to a variety of causes, among which sampling error, analytical error, or physiological ac-

TABLE I

FORMIC ACID FORMATION DURING THE FERMENTATION OF GRAPE MEDIUM (YEASTS 66 AND CHAMPAGNE)

TIME	SUGAR UTILIZED PER 100 ML.		TOTAL VOLATILE ACID FORMED PER 100 ML.		FORMIC ACID PRESENT PER 100 ML.	
	66	CHAMPAGNE	66	CHAMPAGNE	66	CHAMPAGNE
	m. mol.	*m. mol.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*
0 hr.					0.08	0.08
12 "	4	4	0.04	0.01	0.15	0.07
24 "	7	11	0.29	0.11	0.26	0.08
36 "	20	12	0.49	0.49	0.26	0.08
48 "	41	15	0.56	0.68	0.07	0.10
59 "	49	18	0.62	0.83	0.05	0.11
72 "	52	25	0.59	0.98	0.07	
83 "	65	25	0.63	1.01		0.11
96 "	75		0.62	1.09	0.18	
108 "	76	43	0.65	1.17	0.10	0.06
120 "	82	58	0.62	1.21	0.11	0.07
6 days	89	63	0.65	1.21	0.07	
7 "	94	77	0.69	1.26	0.09	0.06
8 "	96	81	0.70	1.27	0.05	
9 "	99	83	0.68	1.27	0.07	0.04
11 "	104	101	0.70	1.33	0.05	0.06
14 "	109	104	0.76	1.32	0.05	
17 "	109	108	0.73	1.30	0.03	0.05
21 "	113	112	0.74	1.33	0.03	
26 "	114	113	0.72	1.33	0.04	
31 "	114	114	0.73	1.35	0.03	0.03
38 "	115	0.70	1.36	0.05	0.05	
46 "	115	115	0.79	1.37	0.05	
60 "	115	115	0.76	1.38	0.03	
75 "	115	115	0.75	1.34	0.01	0.04

tivity of the yeast are to be considered. At the time of sampling, the fermentation mixture was rotated gently to produce a uniform sample without unduly aerating the fermentation. This might not have been a sufficient precaution to avoid slight fluctuations in the experimental results due to sampling errors. Since the quantity of formic acid present in any of the samples was very small, any errors in the analyses would have been magnified when the results were calculated. If the possible sources of error are disregarded, the data in table I indicate that there was a slight formation of the acid in the early stages of fermentation followed by utilization. This was much more marked for yeast 66 than for any of the other yeasts. The actual amount of formic acid found at any particular stage of the fermentation probably represents a balance between formation and decomposition with slight accumulation in the medium in the initial stages of the fermentation; that this was followed by utilization of the acid, is in agreement with the results of FRANZEN and STEPPUHN (1, 16).

In figure 1 the solid lines show these data obtained from the grape juice

medium. The total volatile acid formed, and formic acid present, are plotted against the sugar utilized. For yeast 66 both these curves are shown, while for Champagne yeast only the formic acid curve is shown.

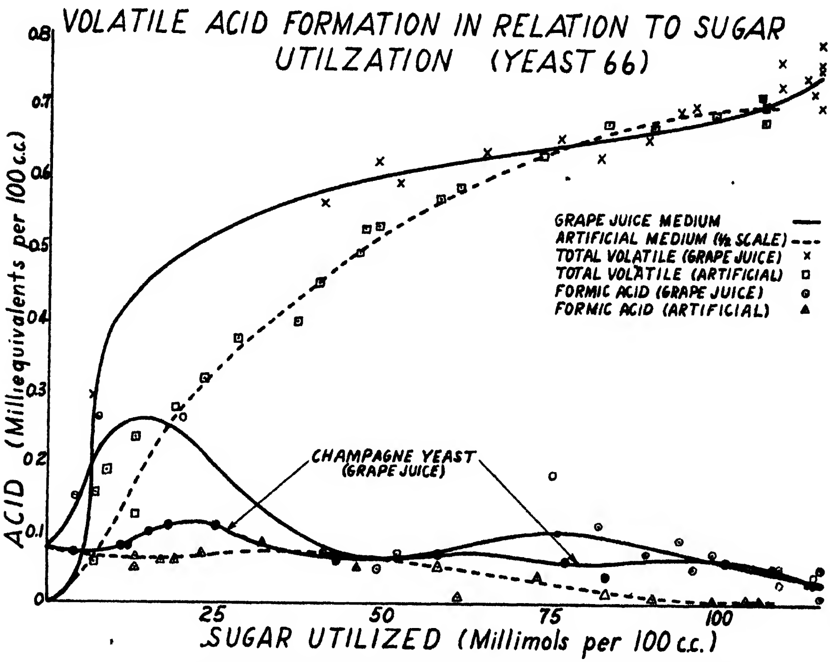

FIG. 1. Volatile acid formation in relation to sugar utilization (yeast 66).

FORMIC ACID FORMATION DURING FERMENTATION OF THE ARTIFICIAL MEDIUM

Seven lots of the artificial medium having 106 milli-mols reducing sugar and 0.15 milli-equivalents formic acid per 100 ml. (pH 4.38) were fermented by the seven yeasts and samples were withdrawn as in the grape juice experiment. In table II, the analyses of the respective samples withdrawn from the fermentation by yeasts 66 and Champagne are summarized. In figure 1 the data for yeast 66 are plotted at half scale (broken lines).

The pH of the artificial medium dropped rapidly and continuously from near pH 4.0 to somewhat below pH 3.0; that of the grape medium remained nearly constant (about 3.5). Total volatile acid production was considerably higher in the artificial medium than in the grape medium. Its rate of accumulation was lower but this may be correlated with a lower rate of sugar utilization. Here, as in the grape medium, most of the volatile acidity had accumulated when the sugar content had been reduced to half.

The formic acid data in table II and figure 1 show that the initial artificial medium contained more formic acid than was present at any subse-

TABLE II

FORMIC ACID FORMATION DURING THE FERMENTATION OF ARTIFICIAL MEDIUM (YEASTS 66 AND CHAMPAGNE)

TIME	SUGAR UTILIZED PER 100 ML.		TOTAL VOLATILE ACID FORMED PER 100 ML.		FORMIC ACID PRESENT PER 100 ML.	
	66	CHAMPAGNE	66	CHAMPAGNE	66	CHAMPAGNE
	m. mol.	*m. mol.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*
0 hr.	......	......	......	......	0.15	0.15
24 "	3	0	0.06	0.09	......	......
48 "	7	5	0.21	0.27	......	......
60 "	13	5	0.25	0.34	0.13	......
72 "	7	9	0.31	0.42	......	0.11
84 "	9	15	0.38	0.54	......	......
96 "	13	18	0.46	0.62	0.10	0.11
108 "	17	22	0.48	0.75	0.12	0.11
120 "	19	23	0.55	0.79	0.12	......
6 days	23	33	0.63	0.95	0.14	0.12
7 "	28	41	0.74	1.16	......	......
8 "	32	50	0.79	1.27	0.17	0.13
9 "	35	56	0.90	1.38	......	0.05
10 "	46	64	0.99	1.40	0.10	0.04
11 "	47	72	1.06	1.52	......	0.04
12 "	49	80	1.07	1.48	......	0.02
13 "	58	87	1.13	1.63	0.10	0.01
14 "	61	93	1.18	1.52	0.02	......
16 "	73	101	1.26	1.63	0.08	0.01
18 "	83	103	1.37	1.60	0.03	......
20 "	90	104	1.34	1.62	0.02	......
23 "	99	104	1.37	1.62	0.01	......
27 "	104	104	1.40	1.60	0.01	......
32 "	106	104	1.42	1.59	......	0.00
38 "	106	104	1.36	1.61	0.01	......
45 "	106	104	1.40	1.62	0.01	0.00

quent stage of the fermentation. Thus any production of this acid by the yeast during fermentation was masked by greater utilization of it. All seven strains of yeast gave very similar results with respect to formic acid production in this medium.

EFFECT OF CARBON AND NITROGEN SOURCE UPON FORMIC ACID FORMATION

In order to determine what effect, if any, carbon source and nitrogen source had upon formic acid formation several natural and artificial media were fermented for one month with three of the strains of yeast (numbers 66, 2368, and Champagne). The natural media were white and red grape juice, diluted white grape concentrate, and wort. For the artificial media, the basic medium was modified. In one series the carbon source was always 200 grams of cerelose per liter and the nitrogen source was varied so that the total amount of nitrogen added was always the same. In another series the nitrogen source was always three grams of ammonium sulphate per

liter and the carbon source was varied, always using 200 grams per liter of the respective sugar. The formic acid contents of the final samples of these fermentations, one month after inoculation, ranged from 0.0 to 0.19 milli-equivalents per 100 ml. With the exception of the β-alanine, cerelose, and red grape juice fermentations the formic acid contained in these final media was negligible (0.01 to 0.06 milli-equivalents per 100 ml.). In β-alanine the final amount of formic acid was almost identical with that initially present. This is probably due to the failure of the yeasts to ferment in this medium, and indicates that formic acid is normally utilized to a greater extent than it is produced in active fermentation. In red grape juice the final quantities of formic acid were somewhat higher than in the other media, (averaging 0.15 milli-equivalents per 100 ml.). This was close to the initial amount present.

THOMAS (**38**), while believing that formic acid was formed from sugar, found that its accumulation was considerably increased in media containing amides. In these experiments, the only amide used was asparagine and the results with it do not show any such relationship.

FORMIC ACID UTILIZATION

The evidence from previous experiments indicated that any formic acid which might have been formed by these yeasts was destroyed almost as rapidly as it was formed. If there was any accumulation of it in the media it occurred very early in the course of fermentation and was followed by more or less complete destruction. Since the quantities of formic acid found were small in any of the media, further experimental evidence was required to determine whether this acid was actually destroyed by these yeasts. Therefore, a series of media was prepared having the following compositions:

(a) Basic artificial medium. Control.
(b) Basic artificial medium plus 0.1 per cent. formic acid.
(c) Basic artificial medium without cerelose, plus 0.1 per cent. formic acid.

To media (b) and (c) sufficient sodium hydroxide solution was added to bring their pH to that of medium (a) (*i.e.*, 3.45). Again only three strains were used—66, 2368, and Champagne. In order to insure vigorous cultures, these media were inoculated with five per cent. by volume of cultures of the respective yeasts which had grown on grape juice for two successive three day periods. One series of these media was analyzed immediately after inoculation for pH, total volatile acid, and formic acid. Another series of duplicate samples was analyzed after one month of fermentation. The BRIX degree of these final samples was also recorded as an indication of the approximate quantity of residual sugar. The results of these initial and final analyses are summarized in table III.

TABLE III

FORMIC ACID UTILIZATION

YEAST	MEDIUM	FORMIC ACID PRESENT PER 100 ML.		FORMIC ACID UTILIZED	TOTAL VOLATILE ACID PRESENT 100 ML.		TOTAL VOLATILE ACID FORMED PER 100 ML.	FINAL BRIX
		INITIAL	FINAL		INITIAL	FINAL		
		m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	
66	(a)	0.12	0.14	–.02	0.11	1.54	1.43	< 0°
C*	(a)	0.12	0.10	.02	0.11	1.48	1.37	< 0°
2368	(a)	0.12	0.10	.02	0.11	0.77	0.66	2°
66	(b)	1.48	1.13	.35	1.16	1.34	0.18	< 0°
C	(b)	1.48	1.29	.19	1.16	1.99	0.83	< 0°
2368	(b)	1.48	1.30	.18	1.16	1.86	0.70	4.0°
66	(c)	1.41	1.55	–.14	1.15	1.00	–.15	
C	(c)	1.41	1.38	.03	1.15	0.92	–.23	
2368	(c)	1.41	1.38	.03	1.15	1.00	–.15	

* C = Champagne.

These data show that there was no significant utilization of formic acid in the absence of sugar. In the presence of sugar there was evidence for slight utilization by all three strains studied. THOMAS (39) also observed that yeast press juice can ferment formate only in the presence of sugar.

The effect of formic acid in the medium upon total volatile acid formed, *i.e.*, upon acetic acid formation, is of interest. With yeast 66 in the presence of formic acid, acetic acid formation was markedly suppressed; only a little over ten per cent. of the amount formed in the control experiments was found. With Champagne yeast only about sixty per cent. as much acetic acid was formed in the presence of formic acid as in its absence. With yeast 2368, on the other hand, there was no inhibition of acetic acid formation by formic acid. The BRIX degrees of these same samples show that this inhibition was not due to a general retardation of fermentation, but rather to a physiological difference among the strains of yeast.

EFFECT OF OXYGEN SUPPLY AND pH UPON FORMIC ACID FORMATION

In order to obtain some information on the effect of oxygen supply upon formic acid formation, fermentations of the artificial medium were conducted in the presence of a limited oxygen supply; *i.e.*, in cotton-plugged liter bottles, and under CO_2 pressures (using mercury bungs on the fermentation bottles). After the fermentations had been allowed to go on for eight weeks the samples were analyzed. The total volatile acidity was about ten per cent. higher in the fermentations conducted in the presence of a limited oxygen supply than in the corresponding ones conducted under carbon dioxide pressure. In the former the amounts formed ranged from 1.71 to 2.37 milli-equivalents per 100 ml., while in the latter they ranged from

1.36 to 2.25. There was no net formation of formic acid in this experiment since the final media all contained less than the initial medium.

In all the experiments thus far discussed, the presence of formic acid in the original media obscured the results on formic acid formation. Small quantities of formic acid occurred normally in pasteurized grape juice and also in the yeast juice used as a growth stimulant in the artificial media. It was then found possible to prepare an artificial medium free of formic acid by replacing the yeast juice with a rice bran concentrate (Galen's Vitamin B concentrate). Duplicate lots of this medium were adjusted to pH 2.5, and pH 6.7 with phosphate buffers. The control media had an initial pH of 4.8. Two fermentations of the control medium were provided with mercury bungs, two were cotton stoppered, and two were aerated by attaching them to a water aspirator and filtering the incoming air through sterile cotton. Yeast 66 was the only organism used in these experiments. The results of this experiment are summarized in table IV. They show that no formic acid was formed in these media except in the medium having an initial pH of 2.5; in this 0.08 milli-equivalents per 100 ml. were found. Although the quantities of formic acid found by Esau (9) to be formed by yeast 66 were three or four times as great as the quantities formed in these experiments, the results are in agreement in that more of the acid was formed in a medium at a low pH than at a high pH.

TABLE IV

Effect of aeration and pH upon acid formation by yeast 66

Amount of aeration	Initial pH	Final pH	Formic acid present 100 ml.	Acetic acid present per 100 ml.	Final Brix
			m. eq.	m. eq.	
Cotton stopper	4.8	2.6	0.002	1.65	0.0°
Hg bung	4.8	2.55	0.003	1.61	0.0°
Aerated	4.8	2.34	0.004	3.17	5.4°
Cotton stopper	2.5	2.48	0.08	1.20	8.6°
Cotton stopper	6.7	3.57	0.002	0.98	0.0°

After an initial medium free of formic acid had been developed, further experiments were conducted using the basic artificial medium with Galen's Vitamin B concentrate added for growth stimulation. These experiments were made to test certain possible precursors of formic acid. The substances which were added to the respective lots of media were 0.2 per cent. U.S.P. tannic acid, 0.2 per cent. grape tannin, 0.5 per cent. peptone, 0.3 per cent. dihydroxyacetone, 0.2 per cent. sodium pyruvate, 0.2 per cent. pyruvic acid. Tannic acid and tannin were chosen because of their abundant pres-

ence in red grape juice, and the likelihood that this might explain the relatively high formic acid yields found in the red grape media. Peptone was chosen because the media used by ESAU (9) which had given higher formic acid yields than those observed by us, had contained this nitrogen source. Dihydroxyacetone and pyruvic acid or pyruvate were chosen because they appeared to be the most logical three carbon precursors of formic acid. All these media were inoculated in duplicate with yeast 66, in eight-ounce bottles containing 150 ml. of medium both with cotton stopper and with mercury fermentation bungs. Another set of duplicate fermentations for each respective substance was made by allowing the control medium to ferment to approximately half its original sugar content, and then adding an appropriate amount of the respective substance aseptically. Each substance was tested both in the presence and absence of sugar. All these media were allowed to ferment for one month and were then analyzed for formic acid, total volatile acid, and pH. No formic acid was found to be formed from any of these precursors. Tannic acid and tannin in the medium appeared to interfere with the mercury procedure of formic acid determination by giving a precipitate, but more of this precipitate was found in the initial media than in the final fermented samples. This might explain the high results obtained on red grape juice media.

The majority of the experiments thus far discussed have indicated that no formic acid is present in the final completely fermented medium. It was thus still desirable to explain why the earlier investigators including ESAU (9) and JOSLYN and DUNN (20), who had used the same strains of yeast in pure culture, had been able to report from 0.35 to 1.06 milli-equivalents of formic acid per 100 ml. of medium, while the amounts found in the final media of any of our present experiments had ranged from 0 to 0.18 milliequivalents. It seemed possible that differences in method of determination might help to explain these differences in results. These other investigators had used the DYER (6) distillation procedure for their formic acid determinations. The DYER method, like other fractional steam distillation procedures is most accurate for samples containing 0.5 per cent. or over of volatile acid. When applied to samples containing 0.1 per cent. of volatile acids, errors in concentrating the original steam distillate to a suitable volume might be high; owing particularly to entrainment of relatively less volatile acids such as lactic.

After determining the DYER distillation constants for solutions containing 0.1 per cent. of acetic or formic acids, distillates of several of the samples on which formic acid had been determined by mercury precipitation, were prepared and analyzed by the DYER procedure. One-hundred-ml. portions were steam distilled until 900 ml. of distillate was obtained; this was neutralized and concentrated to less than 200 ml., brought to pH 3 by addition of

sulphuric acid, and distilled at constant rate under previously calibrated conditions. Distillation constants were obtained and the former acid content calculated. The data shown in the first five columns of table V indicate

TABLE V

COMPARISON OF RESULTS OF FORMIC ACID ANALYSES BY DYER DISTILLATION AND BY MERCURY PRECIPITATION. RESULTS EXPRESSED IN MILLIEQUIVALENTS PER 100 ML.

SAMPLE	TOTAL VOLATILE ACID	ACETIC ACID BY DYER	FORMIC ACID BY DYER	FORMIC ACID BY HG	LACTIC ACID
1	0.42	0.23	0.19	0.14	
2	1.01	0.87	0.14	0.03	1.27
3	1.77	1.45	0.32	0.13	0.88
4	0.93	0.74	0.19	0.01	1.2
5	1.41	1.05	0.36	0.01	1.3

that except for sample no. 1, which was unfermented grape juice, the DYER procedure gave considerably higher results for formic acid than did the mercury-precipitation method.

The presence of lactic acid in the original prepared distillate was thought to be responsible for these low values since lactic acid would decrease the distillation constant. Lactic acid was known to be present in the samples which were being studied, and measurable quantities of lactic acid are known to be carried over by entrainment in the steam distillate used for volatile acid determinations (**34**) It was found that the lactic acid, when present with acetic acid, in the same relative concentrations as were contained in our samples, decreased the distillation constants sufficiently to account for the high "apparent" formic acid values. The last column in table V, which gives the lactic acid contents of the respective samples, indicates that the sample containing lactic acid showed the greatest discrepancy between the formic acid found by the two methods. There was no quantitative correlation, however, between the concentration of lactic acid present and the magnitude of the discrepancy. Samples low in volatile acid content and relatively high in lactic acid content apparently cannot be accurately assayed by the DYER distillation method.

It may be concluded that formic acid is not a final by-product of alcoholic fermentation of sugar by these strains of wine yeasts. Data on formic acid content obtained by the current procedures when unconfirmed by specific qualitative tests are not significant.

Summary

1. The formation of formic acid in the fermentation of natural and artificial media was investigated under various conditions.

2. During the course of fermentation of grape juice a very slight accumulation of formic acid occurred in the early stages of the fermentation, but this was followed by an apparent utilization of the acid.

3. During the course of fermentation of an artificial medium containing yeast juice, the original medium contained more formic acid than was found at any subsequent stage. The utilization of this acid was greater at all stages of the fermentation than was formation.

4. A variety of carbon sources and nitrogen sources had little effect upon formic acid formation by these organisms. All the fermented media except red grape juice contained negligible quantities of it.

5. No utilization of formate occurred in the absence of sugar, while about 20 per cent. of the available formate was utilized in the presence of sugar.

6. Negligible quantities of formic acid were formed under all degrees of aeration which were used—varying from continuous aeration to fermentation under mercury bungs.

7. The pH apparently influences formic acid formation. A small amount is formed in a medium having an initial pH of 2.5; none is formed at higher pH values.

8. No formic acid was formed by yeast 66 from tannin, tannic acid, peptone, dihydroxyacetone, pyruvic acid, or sodium pyruvate.

9. Lactic acid present in the samples analyzed for formic acid was found to interfere with the determination of the latter by the Dyer fractional steam distillation procedures.

10. Formic acid is not an important end product of alcoholic fermentation of sugar by these wine yeasts.

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SUCCINIC ACID AS A METABOLITE IN PLANT TISSUES

George W. Pucher and Hubert Bradford Vickery

(WITH ONE FIGURE)

Introduction

The preparation of succinic acid by the dry distillation of amber is a process that was known to the alchemists and was clearly described by Agricola as early as 1550 (**12**). The true nature of the substance was probably first recognized by Lemery in 1675, who called attention to its relation to the so-called plant acids in his celebrated "Cours de chimie" (**15**). Berzelius (**5**) determined its elementary composition in 1815 and, during the next few decades, succinic acid was studied by many investigators (**16**); new sources were found and its presence both in plant and in animal tissues was recorded. Of the observations on plant tissues, those of Zwenger (**29**) (who identified succinic acid in the leaves of *Artemisia absinthium* in 1843) and Köhnke (**11**) (who obtained it from the leaves of two species of Lactuca in 1844) are the earliest, according to the critical review of Franzen and Ostertag (**9**), that furnish satisfactory evidence of the identity of the substance.

Franzen and Ostertag pointed out that, at the time of their study (1923), succinic acid had been unequivocally identified in only ten species of plants, and only a few additional reports have appeared since (**4, 22**); in none of these cases have quantitative determinations of the acid been made. There is, however, an extensive literature on the occurrence of succinic acid as a product of the metabolism of lower organisms, particularly as a by-product of yeast fermentation (**8**). Its presence in wines and in decomposing fruits has long been recognized and analytical methods for application in this field have been developed (**6, 10, 20**). The work of Ehrlich suggests that succinic acid arises from the oxidation of glutamic acid during yeast fermentation, a reaction with some analogies to the formation of amyl alcohol from leucine under the same conditions; Neuberg and Cappezzuoli (**17**) have shown, however, that succinic acid may be produced by the reductive deamination of aspartic acid through the action of putrefactive bacteria.

Interest in succinic acid as a metabolite concerned in the normal processes of cell respiration has been greatly stimulated in recent years by the work of Szent-Györgyi and his collaborators (**1, 2**) and of Krebs (**14**) who have shown that the oxidation of carbohydrates in certain animal tissues can be described in terms of a sequence of enzymatic oxidation and reduction reactions in which a series of organic acids, most of which are familiar components of plant leaf tissues, are concerned. Among these is succinic acid,

the oxidation of which to malic acid by an enzyme present in muscle (**21**) was demonstrated by BATTELLI and STERN (**3**) in 1911. Although no conclusive evidence has yet been obtained that the respiration of plant tissues can be explained in a similar manner, CHIBNALL (**7**) has pointed out that there is considerable data in the literature that may be interpreted, at least in part, in this way; additional evidence has been provided by recent studies of the metabolism of rhubarb leaves by the authors (**24, 27**). It appears that the respiration of leaf tissue may, however, involve substrates in addition to carbohydrates (**23**) and, in any case, the process is doubtless more complex than the system that has been demonstrated to operate in pigeon breast muscle and a few other animal tissues (**13**). All speculations, however, that try to account for the production of carbon dioxide by living tissues involve the decarboxylation of organic acids as the step whereby this substance is finally produced. Regardless of the details of the enzymatic processes involved, therefore, organic acids play a dominating rôle in the metabolism of carbon, and it is obvious that the study of the behavior of these substances in living plant tissue is a promising line of approach to an ultimate understanding of the chemical reactions that underlie the phenomena observed.

The determination of succinic acid

A new method to determine succinic acid in plant tissues has been described in a previous paper (**18**). The organic acids are removed from the dry powdered tissue by exhaustive extraction with ether in the presence of a slight excess of sulphuric acid. The acids are transferred to aqueous alkali, the solution is acidified, filtered, and then warmed with an excess of potassium permanganate at faintly acid reaction. All common organic acids other than succinic are destroyed by this reagent. The succinic acid is again extracted with ether, traces of contaminants are removed by oxidation with a minute quantity of nitric and hydrochloric acid, and the completely dry residue is treated with acetyl chloride whereby succinic acid is converted into succinic anhydride. This substance is then condensed with *p*-toluidine in toluene solution to the insoluble succinyl-*p*-toluide which is weighed and recrystallized for identification by means of the melting point and crystalline form. The method permits the determination and positive identification of succinic acid with a precision of $\pm$ 5 per cent. for a single determination when quantities from 1 to 20 mg. are taken. The mean recovery of known amounts of acid in a series of replicate determinations may be expected to be about 99 per cent. The only known interfering acid likely to be encountered in plant tissue analysis is α-ketoglutaric acid which would accompany the succinic acid up to the oxidation step and is itself oxidized by permanganate to succinic acid. Accordingly the results of the method

include this substance if present. So far as is known, however, α-ketoglutaric acid is rarely present in plants save in small amounts and in any case, according to current views, it is closely related to succinic acid in metabolism.

Succinic acid in plant tissues

It is clear from the foregoing that the distribution of succinic acid in green plants is still largely unknown and there is practically no information available with respect to the relative quantities normally to be found. An examination was accordingly made of the following plant tissue samples; most of these had been previously analyzed in this laboratory for organic acids in connection with other studies.

Beet: variety Detroit Dark Red; harvested Sept. 28, 1934, 105 days from planting; described by VICKERY, PUCHER, and CLARK (**25**). Plants treated with ammonium sulphate during last 8 days of growth and thereby greatly enriched in glutamine content.

Buckwheat: plants grown in water culture with nitrate as the only source of nitrogen; harvested May 1, 1937, 27 days from transplantation of the seedlings; described by PUCHER, WAKEMAN, and VICKERY (**19**).

Bryophyllum: leaf samples from greenhouse plants grown in 1939 in sand culture with nitrogen supplied respectively as nitrate alone and as ammonium nitrate.

Maize: plants grown in soil in greenhouse; samples collected in March, 1935.

Tobacco: seedlings of *N. rustica* var. Brazilia and of *N. tabacum* Rosenberg strain, grown in sand culture with nitrate as source of nitrogen; harvested Oct. 3, 1939, 6 weeks from planting; about 4 cm. high with 3 leaves; entire plant analyzed. Leaf, stalk, and inflorescence samples mostly from plants grown under shade in the field at the Tobacco Sub-station at Windsor, Connecticut. Those dated 1933 have been fully described by VICKERY, PUCHER, LEAVENWORTH, and WAKEMAN (**26**).

Tomato: plants grown in soil in greenhouse in 1935; 4 months from transplantation; flower buds removed as they formed.

Rhubarb: leaf samples collected from field grown plants June 6, 1936; described by VICKERY, PUCHER, WAKEMAN, and LEAVENWORTH (**27**).

The results are given in table I; succinic acid was present in all save possibly the single sample of rhubarb leaf blade tested. In this case, the amount found was below the range of the analytical method. It is particularly significant that succinic acid does not account for any substantial part of the unknown acids of these tissues except for a few of the tobacco plant samples; there are only two cases in which it makes up more than 10 per cent. of the total organic acids. In the extreme case of maize leaves, the unknown acids, that is the organic acids other than malic, citric, and oxalic,

TABLE I

SUCCINIC ACID CONTENT OF PLANT TISSUES

TISSUE	TOTAL ORGANIC ACIDS PER 100 MG. DRY WT.	UNKNOWN ORGANIC ACIDS AS PERCENTAGE OF TOTAL ACIDS	SUCCINIC ACID: PERCENTAGE OF DRY WT.	SUCCINIC ACID: PERCENTAGE OF TOTAL ACIDS	SUCCINIC ACID: PERCENTAGE OF UNKNOWN ACIDS	MELTING POINT OF TOLUIDE
	m. eq.	%	%	%	%	°C.
Beet: tops	199	21.2	0.21	1.8	8.4	173
roots	97	57.7	0.47	8.2	14.3	174
Buckwheat: leaves	329	5.7	0.20	1.0	18.1	174
stems	155	19.9	0.19	2.1	10.5	176
Bryophyllum leaves: nitrate	423	49.7	0.23	0.9	1.9	173
ammonia	314	59.3	0.19	1.0	1.7	175
Maize leaves	118	69.3	0.19	2.7	3.9	176
Tobacco: rustica seedlings	164	38.0	0.14	1.5	3.8	174
Rosenberg seedlings	136	15.1	0.11	1.4	9.1	173
stalk (1935)	114	54.6	0.24	3.6	6.5	174
stalk (1933 K)	83	54.4	0.65	13.3	24.3	175
leaves (1933 G)	236	17.8	0.55	4.0	22.2	174
leaves (1933 B)	182	20.6	0.31	2.9	14.1	175
leaves (1937)	190	33.4	0.21	1.9	5.6	175
inflorescence (1933 H)	104	43.4	0.28	4.6	10.7	174
inflorescence (1933 I)	55	27.2	0.25	7.7	28.3	......
inflorescence (1933 J)	74	55.5	0.80	18.4	33.2	176
inflorescence (1933 K)	53	40.7	0.14	4.5	11.0	......
Tomato: leaves	125	61.6	0.42	5.7	9.3	175
stalks	97	71.2	0.52	9.1	12.7	175
Rhubarb: leaf blade	279	30.4	0.03	0.2	0.6	.....
petiole	495	3.4	0.12	0.4	11.9	173

account for 69 per cent. of the total organic acidity. Succinic acid, however, accounts for only 2.7 per cent. of the total, or less than 4 per cent. of the unknown fraction. This result is of especial interest in connection with the observations of WADLEIGH and SHIVE (**28**); from a study of the buffer index of expressed saps, these authors pointed out the possibility that maize leaves may be unusually rich in succinic acid.

In general, succinic acid makes up from a trace to somewhat less than 1 per cent. of the dry weight of these tissues, and from about 1 to a maximum of 18 per cent. of the organic acidity; in most cases it accounts for less than 5 per cent. of the acidity. From the point of view of relative quantity present, succinic acid is obviously a minor acid constituent. No example has been reported in the literature in which it forms any substantial part of the weight of a plant tissue, as is frequently the case for malic, oxalic, and citric acids, and, were it not for the fact that succinic acid is easily prepared synthetically, it would doubtless rank with such substances as aconitic or quinic acids as one of the rarer plant acids. The present data show, that,

on the contrary, succinic acid is widely distributed, although in small relative quantities, and indicate that it may have considerable importance in metabolism. The fact that so little is found in a given case suggests that the substance is involved in the living tissue in reactions that proceed with some rapidity, no great concentration being necessary in order to upset the equilibrium relationships in favor of the next product in the series of metabolic reactions in which it is presumably involved. The more comprehensive data on the tobacco plant show that succinic acid may vary between wide limits in the amount present in different tissues and at different periods of development of the plant.

Succinic acid in the tobacco plant at different stages of growth

Analyses were also made of a series of samples of tobacco plants that represented, respectively, leaf, stalk, and inflorescence tissue collected at intervals from plants growing in the field under shade in the season of 1933. These samples had been previously analyzed for organic acids, carbohydrates, and nitrogenous components with results that have already been published (**26**); frequent reference will be made in the following to the data of this publication. The results for succinic acid are shown in table II. In the first and second sections of the table, the analyses are calculated in the conventional manner as percentages of the fresh and of the dry weight. These data, when plotted, give curves that show many irregularities, but which indicate that the concentration of succinic acid in the leaf tissue increased to a maximum at 54 days from transplanting of the seedling and subsequently fell rapidly, as the inflorescence developed, to a low and constant value. In the stalk, the concentration of succinic acid when calculated on the fresh basis, remained fairly constant; it dropped rapidly, however, if calculated on the dry basis, until the final sample of mature plants with nearly ripened seed-pods was reached. This sample showed a remarkable enrichment in succinic acid.

The behavior of succinic acid in the inflorescence is particularly striking; at 97 days when the seeds were rapidly ripening, the pods had become greatly enriched and contained, in fact, more than 0.8 per cent. of this acid. In the last sample, taken two weeks later, the succinic acid in the seed-pods had again fallen to a low concentration. Repeated analyses of these samples were made to insure the absence of analytical errors. Reference to the earlier analytical data on the same material (**26**) shows that no other component of the pods for which data were obtained, save the unknown acids, behaved in a similar fashion, although there was a moderate loss of water in the final interval between analyses.

The third section of the table shows the data calculated in terms of milliequivalents per single plant. These results are plotted in figure 1 and, in

TABLE II

SUCCINIC ACID CONTENT OF THE TOBACCO PLANT

DAYS FROM SETTING	19	26	35	40	47	54	61	75	97	110
I. PERCENTAGE OF FRESH WEIGHT										
Leaves	0.035	0.035	0.029	0.038	0.035	0.058	0.057	0.018	0.017	0.019
Stalk	......	......	0.026	0.023	0.020	0.029	0.021	0.029	0.006	0.135
Pods	......	......	......	......	......	......	0.042	0.041	0.189	0.046
II. PERCENTAGE OF DRY WEIGHT										
Leaves	0.330	0.341	0.278	0.353	0.375	0.564	0.537	0.145	0.124	0.125
Stalk	......	......	0.302	0.338	0.258	0.270	0.169	0.190	0.031	0.676
Pods	......	......	......	......	......	......	0.306	0.262	0.819	0.144
III. MILLIEQUIVALENTS PER PLANT										
Leaves	0.030	0.098	0.485	0.958	2.26	4.47	4.18	1.64	1.09	1.21
Stalk	......	......	0.132	0.336	0.958	1.86	1.52	2.68	0.52	13.2
Pods	......	......	......	......	......	......	0.062	0.493	4.75	0.95
Whole plant	......	......	0.617	1.29	3.22	6.33	5.76	4.81	6.36	15.3
IV. PERCENTAGE DISTRIBUTION IN PLANT										
Leaves	......	......	78.5	74.1	70.2	70.6	72.6	34.1	17.1	7.9
Stalk	......	......	21.4	25.9	29.8	29.4	26.3	55.7	8.2	85.8
Pods	......	......	......	......	......	......	1.1	10.2	74.7	6.2
V. PERCENTAGE OF UNKNOWN ACIDS										
Leaves	14.4	9.2	10.2	15.2	18.4	22.4	25.5	7.1	6.0	5.6
Stalk	......	......	8.1	13.8	8.3	8.4	6.1	5.5	1.2	24.8
Pods	......	......	......	......	......	......	10.3	28.0	33.6	11.0

contrast to the percentage data, give relatively smooth curves, the major inflections of which correspond to definite phases in the general course of development of the plant. Considering first the curve for the whole plant, it is clear that succinic acid accumulated rapidly during the preliminary period of growth up to 54 days but thereafter, for the next month, changed but little. During the last two weeks, however, succinic acid increased with remarkable rapidity; the total amount present in the plant was more than doubled. Examination of the other curves shows that this final accumulation was due to the rapid increase of succinic acid in the stalk of the matur-

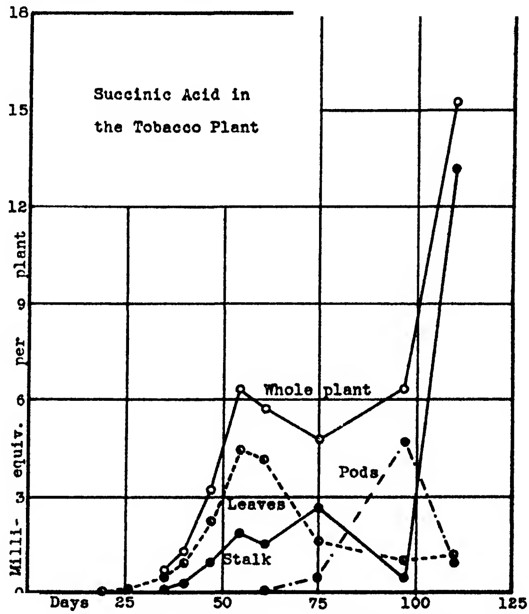

FIG. 1. Succinic acid in the tobacco plant at different stages of growth as measured from the time of transplantation of the seedling. The plants were of the variety known as Connecticut shade-grown and the samples were taken from a normal field crop in the season of 1933.

ing plant, this being far greater than the decrease in the pods during the same period. The apparent cessation of succinic acid synthesis in the plant as a whole throughout most of the period in which the inflorescence was developing recalls the behavior of the citric and oxalic acid observed previously in this same experimental material. There was a marked diminution in the rate of accumulation of these acids at the time that development of the pods began. Malic acid, on the contrary, continued to increase to the end of the period of observation, although more slowly in the last weeks of growth.

The details of the behavior can be best appreciated from the separate

curves for the leaves, stalks, and inflorescence. Succinic acid increased rapidly in the leaves to a maximum at 54 days, but shortly thereafter began to decrease, the final value being about one-quarter of the maximum. In the stalks, the preliminary rate of increase was slower and the maximum was not reached until 75 days; at 97 days the succinic acid had fallen to a very low level, but a phenomenal increase then occurred so that the stalks collected at 110 days contained 13 milliequivalents per plant or about 0.68 per cent. of the dry weight. The inflorescence, which first began to differentiate at about 54 days, had increased by 61 days so that samples could be collected. The ripening process subsequently proceeded rapidly with striking increases in dry weight and particularly in the fat content of the pods. Succinic acid increased up to 97 days and the sample collected at that time was the richest of any tissue hitherto analyzed by the present method. During the later stages of the ripening period, however, succinic acid diminished again to a low level.

In the fourth section of table II are calculations of the distribution of succinic acid in the plant. It is to be noted that, from 35 to 61 days, the proportion of the total succinic acid of the plant present in the leaves underwent very little change in spite of a tenfold increase in quantity. Roughly three-fourths of the acid was found in the leaves at each point of observation. Subsequently, during the period of development of the flowers and seed-pods, the distribution of the succinic acid fluctuated between wide limits although the total amount present did not change materially until towards the end of this process. These figures indicate clearly that succinic acid is a metabolite that is in some manner concerned with the important chemical changes that occur during the reproductive period of the development of the plant.

Discussion

The behavior of succinic acid in the tobacco plant may be considered from at least two different points of view. The wide changes in distribution of a nearly fixed quantity of the acid that occurred during the development of the inflorescence may be considered to furnish an example of transport of the acid from one part of the plant to another according to the demands of the chemical reactions that took place. The diminution of the quantity of acid in the leaves in the interval from 54 to 75 days amounted to 2.83 milliequivalents per plant. During this time 0.82 milliequivalent appeared in the stalk and 0.49 milliequivalent in the pods. There was thus an overall loss of 1.52 milliequivalents of acid. However, in spite of this, migration of the acid may have occurred. In the interval between 75 and 97 days, the leaves lost an additional 0.55 milliequivalent and the stalk 2.16 milliequivalents; meanwhile the pods gained 4.26 milliequivalents. Here there was an

apparent new synthesis of 1.55 milliequivalents of acid. Again disregarding the discrepancy, it is possible to assert that migration of succinic acid took place. But in the last interval between collections, that is between 97 and 110 days, the leaves did not change significantly in succinic acid content, the stalk gained 12.7 milliequivalents and the pods lost 3.8 milliequivalents. Accordingly there was an increase in the amount of succinic acid in the plant as a whole of 8.9 milliequivalents. The increase took place exclusively in the stalk and thus, even if the whole of the succinic acid lost from the pods had migrated into the stalk, it could account for only a small part of the increase. These observations indicate that the idea of translocation as a means to account for the fluctuations of the succinic acid content of the different parts of the plant is at least inadequate. Although nothing has been observed that forbids this as a partial explanation, it is obvious that each of the tissues possesses the capacity to synthesize succinic acid. This capacity is especially pronounced in the stalk during the final period of maturation of the seeds.

A serious objection to the view that succinic acid migrated in substantial amounts out of the developing pod tissue in the last period is that the dry weight, the ether extractives, and the nitrogen in the pods increased materially in this interval (**26**). The increase in the ether extractives, much of which consisted of true fat, was especially striking at this time; there is no doubt, from the behavior of the nitrogen fractions that were examined, that protein synthesis was going on at a rapid rate in the developing seeds. A loss of water from the pods did, in fact, occur; this may well be accounted for simply as the gradual dehydration of the capsules as ripening of the seeds took place; it does not necessarily connote migration of water out of the pods into the stalk.

It seems clear, therefore, that the main stream of translocation at this period in the development of the plant was into, rather than out of, the seed-pods. Accordingly, the argument in favor of the view that translocation accounts for the loss of succinic acid from the pods breaks down in the face of the data already at hand in connection with these particular samples of tobacco plant material.

In the early stages of the growth of the plant, it is clear that synthesis of succinic acid took place in both leaf and in stalk tissue throughout the period up to the beginning of the development of the organs of reproduction, at rates such that the distribution, as between leaves and stalk, remained practically constant. The amount in the leaves at 54 days increased by a factor of 8 over the amount at 35 days; in the stalk in the same interval the amount increased by a factor of 13. Actual growth, as measured by the increase in dry weight of the respective tissues, was by a factor of 3.5 for the leaves and by nearly 15 for the stalk. The increase in succinic acid in

the leaves was thus greater proportionately than the rate of growth, although in the stalk it was of about the same relative magnitude.

The observations suggest that succinic acid is best to be regarded as an active metabolite concerned in the intracellular chemical reactions. As has been seen, there is no need to stipulate that the increases and decreases in the different tissues are the results of transport; rather they appear to reflect the course of reactions which proceed at different rates at different periods in the life of the plant. If succinic acid is related to metabolism in plants in some such manner as that envisaged by CHIBNALL, that is, if it is continuously produced and decomposed through the influence of enzymatic oxidation and reduction reactions that have to do with the respiration, the behavior observed can be reasonably accounted for. The accumulations and disappearances may thus furnish evidence for alterations in the rates of the various reactions that in turn depend upon the chemical nature of the substrates being respired and the demands of the system for energy. Present knowledge of the details of plant cell respiration does not permit precise definition of these chemical reactions and this view is suggested with full realization of its entirely speculative nature. It may serve, however, to stimulate investigation into a more exact description of the functions of the organic acids in plants.

One further point merits attention. It was shown in the earlier discussion of the composition of this series of tobacco plant samples (**26**) that the so-called unknown organic acids (the difference between the total organic acidity and the sum of the malic, citric, and oxalic acids) increased rapidly in the stalks at the end of the period of observation. The increase noted was 14.0 milliequivalents in the interval from 97 to 110 days. Reference to table II, section III, shows that the succinic acid of the stalk tissue increased by 12.7 milliequivalents per plant at this time. Accordingly, the marked change in the unknown acids previously noted is now accounted for nearly exactly as an enrichment of this tissue in succinic acid. Similarly the loss of 5.7 milliequivalents of unknown acids from the pods in the same time interval is accounted for to the extent of 3.8 milliequivalents by the loss of succinic acid from this tissue.

In the fifth section of table II are given calculations that show the proportion of the unknown organic acids previously determined that can now be accounted for as succinic acid. It is apparent from these figures that succinic acid is seldom responsible for any substantial part of the unknown acid fraction. Although in the two cases mentioned above, it can be shown that the *changes* in the unknown acids are largely due to changes in the succinic acid, this is rarely the case. Succinic acid is indeed an important member of the group of previously unknown acids, but our knowledge of the qualitative composition of this group of substances is still extremely

slight. It would be a simple matter to write a list of the acids that might be expected from theoretical considerations to be present in such fractions; this, however, is quite a different matter from the demonstration that each substance listed does in fact occur in them. The present study shows clearly that a satisfactory verification of present-day theoretical views will involve a thorough study of the qualitative and quantitative composition of the so-called unknown fraction of the organic acids of plant tissues.

Summary

Succinic acid is shown to be present in a number of normal plant tissues although the proportion found was in all cases considerably less than 1 per cent. of the dry weight. As compared with malic, oxalic, or citric acid, it is a minor acid consistituent.

A study was made of the behavior of succinic acid during the development of the tobacco plant. The extensive changes in amount and in concentration that this substance undergoes indicate that it may be regarded as one of the more active metabolites of the tissues.

Although no direct support was found for CHIBNALL's view that respiration in plant tissues is to be accounted for in terms of the KREBS citric acid cycle, the observations show that one more of the acids concerned in this scheme of reactions is widely distributed in plants, and that the changes in amount present, at different stages of growth of the tobacco plant, are such as to suggest that succinic acid is involved in the reactions that take place within the cells. This behavior is to be anticipated if some such cyclic series of chemical reactions does indeed lie at the basis of one of the fundamental physiological functions of plant cells.

AGRICULTURAL EXPERIMENT STATION
NEW HAVEN, CONNECTICUT

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BOUND WATER IN PLANT SAP AND SOME EFFECTS OF TEMPERATURE AND NUTRITION THEREON

RAY CALVIN CHANDLER

(WITH FOUR FIGURES)

Introduction

Most plants developing under very favorable conditions for growth are tender and exhibit little resistance to frost damage. Certain plants, however, are capable of developing the capacity to resist freezing and are then said to be hardened. While exposure to low temperature is the principal natural and artificial agency producing such an effect, apparently any condition unfavorable to growth may contribute to some degree of hardening (**5, 19, 31, 32**).

The work of ROSA (**31**) and HOOKER (**16**) indicated that the hardiness of plants was associated with an increase of hydrophilic colloids. NEWTON and GORTNER (**29**) proposed a cryoscopic method for the measurement of such colloids, believing they reflected the amount of water bound by the tissue in such manner as to protect the plant from frost damage. A vast amount of data has accumulated in support of this general view; namely, that colloidal material in the plant may retain water in the liquid state at temperatures well below the normal freezing point and thus protect the plant. The implication here is that hardiness is related to bio-colloids by virtue of their water-retaining capacity and that the water thus retained is held so firmly to colloidal surfaces that it is unavailable for purposes of solution or for ice formation. Such water has been designated as "bound."

The assumption of a correlation between bound water and hardiness in plants elevates the subject to an important position in plant physiology. Since the variation in the response of plants to low temperature is a decisive factor in plant distribution and agricultural practices, the rôle that bound water may play in the temperature effect is a subject of considerable interest.

The principle involved in the determination of bound water by the cryoscopic or other comparable methods is based upon the thermodynamic properties of the solution comprising the sap. It is a well known fact that any solute has a definite effect upon the freezing point depression, vapor pressure lowering, or osmotic value of pure water; and the numerical values of these three colligative properties are interrelated through the applicability of the gas laws to solutions. Few, if any, solutes yield the theoretical values implied by the gas laws but non-electrolytes approach the ideal value. In certain instances, notably in the sap of hardened plants, the freezing point depression has been found to be greater than expected. To account for this

deviation from the expected freezing point it has been assumed that a fraction of the water was bound in such a manner that its thermodynamic properties were lost, the bound water molecules being oriented about the surface of colloidal particles. On this assumption, there is a plausible relation between bio-colloids, bound water, and hardiness.

Two important questions confront the student of this problem: (a), do the data yield a measure of the water bound by colloids as defined above; and (b) has the correlation between bound water and hardiness been supported?

Consideration of the data arouses some doubt that a satisfactory measure of hardiness has been disclosed through bound water measurements (**8, 25, 26, 34**). Excellent support for the concept is provided by the work of GORTNER and GORTNER (**11**) who found that tender plants did not contain bound water while hardened plants indicated the presence of significant amounts. On the other hand, ROSA (**31**) demonstrated hardiness in cabbage plants; but SCARTH and LEVITT (**20, 32**) found hardened cabbage cells did not contain bound water. STARK (**34**) found large amounts of bound water in apple shoots but was unable to distinguish degrees of hardiness. MEYER (**26**) found that the amount of bound water was actually less in hardened pine needles than in unhardened needles. This lack of agreement among workers suggests that the reality of a correlation between bound water and hardiness is still a subject for investigation.

The concept of bound water consisting of oriented water molecules about colloidal particles was discussed in a previous paper (**2**). By means of precise vapor-pressure measurements on dilute gelatin solutions it was shown that the random distribution of water molecules was not decreased by the presence of the colloid. When the electrolyte (potassium chloride) was used as the reference substance[1] in the gelatin solution its effect upon the activity[2] of water was less than anticipated, and the magnitude of the deviation of the vapor pressure lowering was markedly affected by temperature changes. In contrast to the negative deviation observed when the electrolyte was used one finds, however, that the use of non-electrolytes such as glucose and sucrose yield positive deviations for comparable measurements of similar solutions. It was demonstrated also that both positive and negative deviations from the expected freezing-point depression could be produced in non-colloidal solutions. Theoretical considerations and experimental evidence were adduced to show that these deviations might be explained most readily by the special properties of ions and of the non-polar and dipolar non-electrolytes in

[1] Reference substance: a standard solute added to the solution to measure properties of the water.

[2] Activity: the thermodynamic concentration rather than the molar concentration. In this case the vapor pressure lowering was less than would be found for the same concentration in pure water.

aqueous solution. Thus the sign and magnitude of the deviation might be strongly affected by the character of the solutes in the solution and of the reference substance. It was indicated that the deviations often considered as a gauge of the water bound by the colloids really reflected the properties of the components of the complex solution.

This paper deals with a study of the effects of temperature and nutrition upon significant changes in the activity of water in the sap of plants grown under controlled conditions, and of factors responsible for such changes, in the light of the foregoing concept. An evaluation of known osmotically active substances, and of their changes in concentration, is made and compared with the change in activity of water. Cryoscopic measurements for bound water are included.

Experiments were accordingly planned to study: (a), the effect of low temperature and low potassium nutrition on the freezing point depression of wheat sap; (b), the deviation of the freezing point from the expected value when glucose or potassium chloride is added to the sap from plants subjected to low temperature or low potassium nutrition; and (c), the quantitative and qualitative change in solutes as the plants harden.

Material and methods

Physical measurements were made by the freezing point method. A Beckmann thermometer, freezing point tube, air chamber, and insulated ice bath constituted the equipment. Two reference substances, the electrolyte KCl and the non-electrolyte glucose, were chosen as solutes and the thermometer calibrated by increments of these solutes. Molal freezing point depression-concentration curves for both reference substances were prepared from data (**18**) for binary solutions. The deviation of the observed freezing point depression from the measurement expected in binary solution constituted the basis for calculations of bound water.

Chemical determinations were made primarily to ascertain the concentration of substances effective in the change of activity of water in the plant sap.

Sugars were determined by the HARDING and DOWNS (**13**) modification of the Shaffer-Hartmann method on fresh cleared sap.

Amino nitrogen was determined by the VAN SLYKE (**35**) gasometric method immediately after expressing and chilling the sap. The SØRENSEN formol titration method (**33**) was also used to check the gasometric method.

Amide nitrogen and ammonium nitrogen were determined according to the methods outlined by MCCALLA (**23**).

Results have been calculated in mols per 1000 grams of water based on dry weight determinations on samples dried *in vacuo* at 70° C.

The plant material selected for experimental work was the well known Minhardi variety of wheat, *Triticum vulgare*. The suitability of Minhardi wheat for studies on hardiness and bound water has been shown by several

investigators (**1, 10, 24, 28**). DEXTER (**7**) showed that this wheat could be hardened by exposure to cold temperature, and most effectively when the plants were illuminated.

Minhardi wheat was germinated according to the procedure of HOAGLAND and BROYER (**15**) and transferred at the end of seven days to nutrient solution tanks (fig. 1). In order to note the effects of potassium deficiency in

FIG. 1. Minhardi wheat grown in culture solution tank showing typical top and root development at end of the experimental period for unhardened plants.

some of the experiments the nutrient solutions were made up as high potassium (HK) and low potassium (LK) solutions; plants grown in these solutions will be referred to as HK and LK plants. At the beginning of each experiment there was a supply of 600 ml of solution per plant with the following ion concentrations:

ION	EQUIVALENTS $\times 10^4$	
	HK SOLUTION	LK SOLUTION
K	31	1
NO_3	100	100
PO_4	19	19
SO_4	35	35
Ca	51	51
Mg	20	20
Na	0	30

TABLE I

Experiments on expressed sap of Minhardi wheat under various conditions of nutrition and temperature

Experiment	Nutrition	pH	Developmental period		FPD of sap	Deviation		Sugars		Conductivity	Moisture content
			20° C.	0° C.		Glucose	KCl	Hexose	Sucrose		
			days	*days*	°C.	%	%	*mols*	*mols*		
1	HK	5.67	56*	0	1.032	4.3	−1.2	0.136	0.003		0.915
2	LK	5.62	66*	0	0.800	4.0	−2.4				0.924
3	HK	5.84	54†	0	1.049	1.5	−2.4			0.0161	0.904
4	HK	5.65	70‡	0	1.173	4.9	−1.2	0.143	0.000	0.0166	0.866
5	LK	5.65	74*	0	1.072	4.4	0.0	0.065	0.000	0.0275	0.902
6	HK	5.95	47	0	0.981	−0.5	−4.3	0.012	0.000	0.0216	0.934
7	HK	6.10	40	14	1.959	5.6	−2.5	0.077	0.010	0.0264	0.883
8	HK	5.73	51	0	0.936	−0.6	−3.3	0.040	0.000	0.0141	0.931
9	HK	5.70	54	0	1.508	0.8	−3.6	0.165	0.031	0.0295	0.866
10	HK	6.15	54	14	2.130	15.1	−8.3	0.174	0.068	0.0192	0.825
11	LK	5.90	56	0	1.403	7.9	−3.5	0.239	0.000	0.0137	0.883
12	LK	5.85	56	17	2.294	11.9	−8.4	0.293	0.013	0.0154	0.834
13	HK	6.00	39	0	1.130	−0.9	1.2	0.078	0.000	0.0195	0.929
14	HK	6.15	39	22	1.950	5.8	−2.8	0.217	0.022	0.0168	0.833
15	HK	6.00	39	34	2.368			0.156	0.015		0.837

* Grown outside greenhouse; unfavorable, high temperature.
† Grown outside greenhouse; favorable temperature and good growth.
‡ Grown outside greenhouse; second growth following heat injury.

The HK solution furnished satisfactory conditions with respect to nutrition throughout the experimental period. The LK solution distinctly limited growth but apparently produced healthy tissue. Experiments 1 to 5 were preliminary experiments carried on outside the greenhouse. The plants for experiments 6, 8, 9, 11 and 13 (table I) were grown in the greenhouse at 20° C. until time of harvest. Plants for experiments 7, 10, 12, 14 and 15 (table I) were also grown at 20° C. through a developmental period to an age and size comparable to that of the first group; they were then transferred to a cold room at 0° C. where they received illumination from a 500-watt Mazda lamp for 15 hours per day for a period of time previous to harvest as shown in table I.

Normal healthy tissue was ground fine and the sap expressed at a uniform pressure of 70 atmospheres. The fresh expressed sap was chilled and determinations were made of the pH, freezing point depression of the sap before and after solutes were added, sugars, conductivity, and moisture content of the sap. These data are also presented in table I. Buffer curves for experiments 9, 10, 11, 12, 13, 14, and 15 are shown in figures 2 and 3, and formol titration curves for experiments 13, 14, and 15 appear in figure 4.

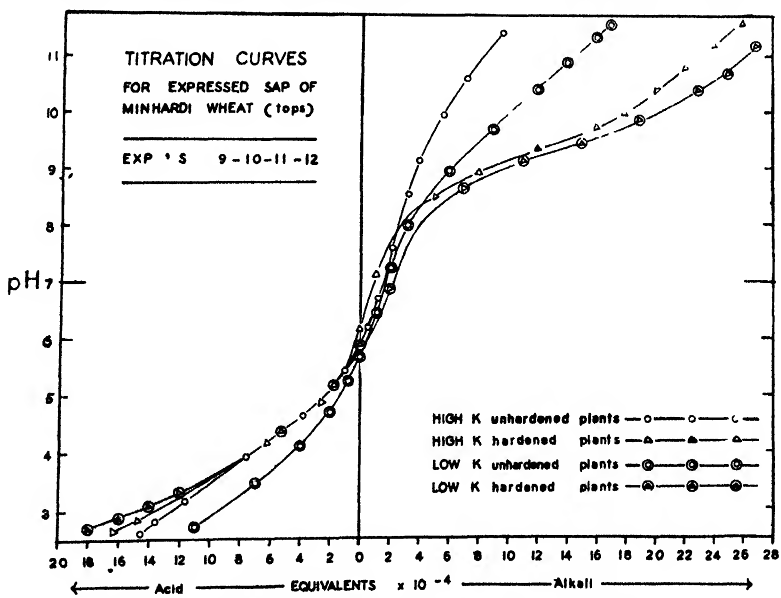

Fig. 2. Titration curves for (a) high potassium plants, hardened and unhardened; (b) low potassium plants, hardened and unhardened.

Important nitrogen fractions which might affect the freezing point of the sap were determined for experiments 13, 14, and 15 as shown by table III. Changes in freezing point depression and in concentration of solutes as plants harden are presented in table IV.

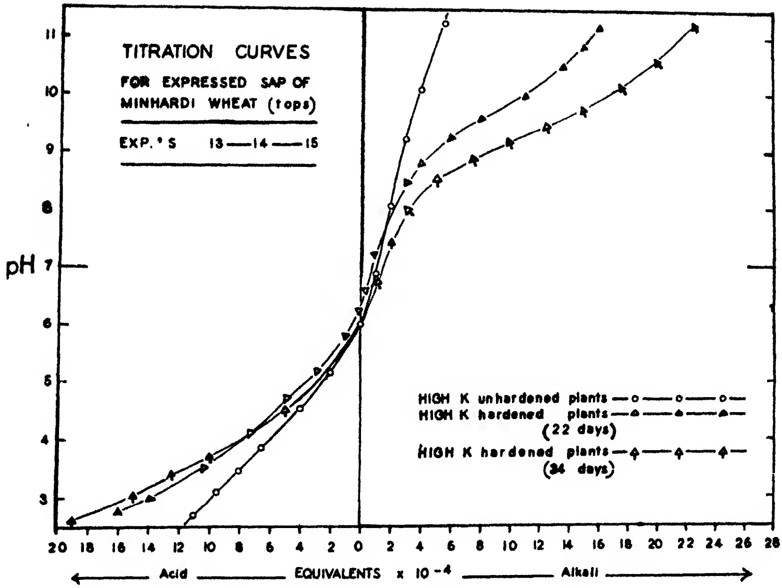

FIG. 3. Change in titration curve (HK plants) with hardening.

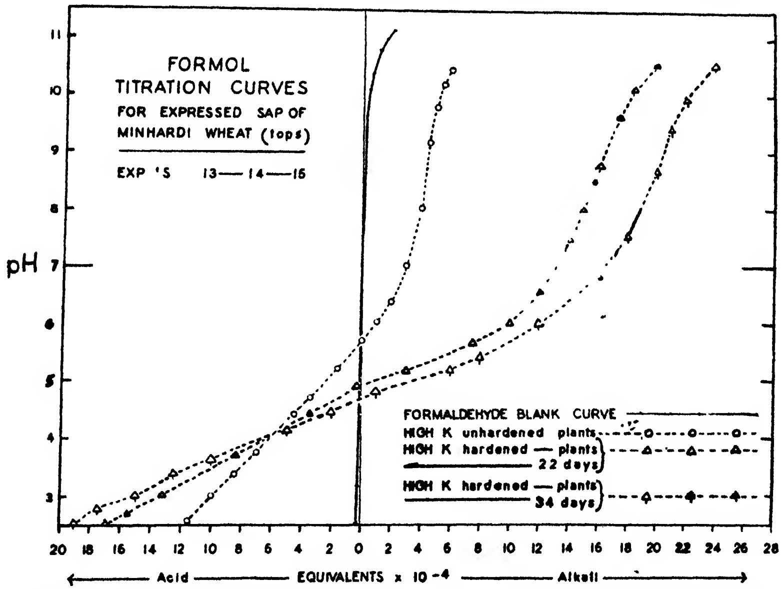

FIG. 4. Formol titration curves showing change with hardening process.

Experiments 6, 7, 8, 9, and 10 were on HK plants. The plants of experiments 7 and 10 were subjected to low temperature for 14 days. Experiments 11 and 12 consisted of LK plants grown for 56 days at 20° C. in one tank.

Plants for experiment 12 were then hardened for 17 days. Plants for experiments 13, 14, and 15 were all grown for 39 days in one large tank under very favorable conditions for growth with respect to temperature and nutrition. At the end of this period, plants for experiment 13 were analyzed and 240 plants of the same lot were transferred to the cold room. After 22 days' exposure the plants for experiment 14 were analyzed and 12 days later (after 34 days of cold exposure) plants of experiment 15 were examined.

Results

The opposing results obtained in freezing point determinations by the use of electrolyte and non-electrolyte as reference substances for the measurement of deviations is fairly consistent throughout (table I). The general effect of unfavorable environment due to high temperature, low temperature, or deficient potassium nutrition is reflected in deviations which are often attributed to bound water. On the other hand, plants grown under favorable conditions (table I, exps. 6, 8, 9, 13) showed negligible deviations when glucose was used as the reference substance. When wheat plants were subjected to cold temperature, the sugars increased (compare expts. 7 and 8, 10 and 9, 12 and 11, 14 and 13). After 22 days' exposure to cold the sugar content began to decline (expts. 14, 15); after 34 days of exposure, however, it still exceeded that in the unhardened plants. The freezing-point depression increased with hardening of the plants.

Table II presents the factors contributing to the freezing-point depression in a number of experiments. These experiments are divided into two groups. The first group contains data from those experiments in which the deviations were negligible while the second group deals with those experiments in which

TABLE II

SUGARS PLUS ELECTROLYTES AND "OTHER CAUSES" AS FACTORS IN PRODUCTION OF FREEZING POINT DEPRESSION

EXPERIMENT	NUTRITION	TREATMENT	FPD TOTAL	FPD* DUE TO SUGARS AND ELECTROL.	FPD OTHER CAUSES	DEV. GLUCOSE
			°C.	°C.	°C.	%
6	HK	Unhardened	0.981	0.553	0.428	−1.2
8	HK	"	0.956	0.421	0.535	−0.7
9	HK	"	1.508	1.135	0.373	0.8
13	HK	"	1.130	0.624	0.506	−0.9
7	HK	Hardened	1.959	0.832	1.127	5.6
10	HK	"	2.130	0.919	1.211	15.1
14	HK	"	1.950	0.854	1.096	5.8
11	LK	Unhardened	1.483	0.781	0.702	7.9
12	LK	Hardened	2.294	0.947	1.347	12.3

* Calculated as mols sugar × 1.86 plus mols electrolyte (as KCl) × 3.40.

marked deviations were observed. It will be noted that the freezing-point depression of the first group is not completely accounted for by the sugars and electrolytes. In spite of considerable differences in the total freezing-point depression of these members, the margin due to "other causes" listed in column 6 is fairly constant, amounting to some 0.4 to 0.5° C. When one compares the two groups it is found that the portion of the freezing-point depression due to "other causes" is some two or three fold greater in the second group.

Interpretation of the foregoing data may be made by aid of figures 2, 3, and 4. Figure 2 shows titration curves for experiments 9, 10, 11, and 12. There is a strong shift between pH 8 and 9 in the curves for sap from plants subjected to low temperature, low potassium nutrition, or a combination of these treatments. Figure 3 also presents titration curves for experiments 13, 14, and 15 in which time of cold exposure is correlated with the amount of alkali required for the titration. Inspection of these curves suggests that they are titration curves for amino acids. Figure 4 consists of formol titration curves for experiments 13, 14, and 15 and provide a measure of the amino acid concentration according to the SØRENSEN technique (**33**). Determinations of the amino acid concentrations were also made by the VAN SLYKE gasometric method (**35**). The relatively large amounts thus disclosed indicate that the amino acid concentration is an important factor contributing to "other causes" in the second group in table II.

Amino acids and other soluble nitrogen fractions which might contribute to the freezing point depression are listed in table III. In table IV are shown

TABLE III

SOLUBLE NITROGEN FRACTIONS. EXPERIMENTS 13, 14 AND 15

EXPERIMENT	TREATMENT	AMINO N		AMIDE N	NH_4-N
		VAN SLYKE	SØRENSEN		
		mols	*mols*	*mols*	*mols*
13	Unhardened	0.052	0.052	0.0046	0.0070
14	Hardened	0.369	0.288	0.0283	0.0054
15	Hardened	0.542	0.348	0.0550	

the changes observed as the plants hardened, with respect to sugars, soluble nitrogen, and freezing-point depression. The fourth column gives the calculated value for the change in freezing-point depression that should be due to the sum of the changes in sugars and soluble nitrogen fractions. Complete data for the changes in electrolytes is not available but would have little effect. It is shown however that the changes in freezing-point depression

closely parallel the changes in solute concentration without resort to a hypothesis for bound water. Cryoscopic measurements on the sap of these same plants yielded a large *positive* deviation when glucose was used as the reference substance, while potassium chloride showed a large *negative* deviation.

TABLE IV

CHANGES IN THE FREEZING POINT DEPRESSION, SUGARS AND SOLUBLE NITROGEN AS PLANTS HARDEN

EXPERIMENT	FREEZING POINT DEPRESSION	CHANGE IN FREEZING POINT DEPRESSION	CALC. FPD* DUE TO CHANGE IN SOLUTES	CHANGE IN SOLUTES PER LITER	
				SUGARS	SOL. NITROGEN
	°C.	°C.	°C.		
13	1.130	0.000	0.000	0.000	0.000
14	1.950	0.820	0.930	0.161	0.339
15	2.368	1.238	1.174	0.098	0.533

* Calculated in °C. (mols solute × 1.86°).

Discussion

The term bound water has been applied indiscriminately to a wide range of phenomena extending from studies of non-vacuolate cells (**22**) and seeds (**21**) at low temperature to phenomena observed in higher plants at temperatures not far below the freezing point of water. Low temperature studies are essentially studies of colloids at low water content while, at the higher temperatures, the water content of the tissue is high and the sap is complex in composition. As was pointed out previously (**2**), it is justifiable to distinguish two classes of phenomena with respect to water relations in colloids: first, those that occur at low water content in which the energy changes arise through irreversible processes; and, second, phenomena occuring at temperatures above the eutectic temperatures of the solutes where free energy changes are due to reversible processes. It is the latter class of phenomena that is considered in this and many other studies on bound water and hardiness in plants.

The question arises as to the justification for the assumption (a) that an unusual value for a thermodynamic property of water in plant sap indicates bound water and (b) that hardiness is the result of this phenomenon. The foregoing experiments show that the quantitative changes in the concentration of solutes in sap during the process of hardening accounts quite satisfactorily for the change in freezing point depression. Moreover, the qualitative changes in solutes would be expected to cause deviations in the freezing point depression such as are obtained by use of the cryoscopic method.

The increase in total sugars, the accumulation of sucrose, the development of amino acids, and the positive deviation in freezing-point depression when glucose is added to the sap, are all characteristic responses of many plants that harden when subjected to cold temperature. The results reported here are in general agreement with other investigations with the exception of the larger amounts of amino acids reported. The evidence that the deviations observed in sap from hardened plants may be either positive or negative by proper choice of a reference substance is new and striking. Since there is no need to postulate bound water, and it is difficult to apply the former concept of bound water to both positive and negative deviations in the same solution, it is more suitable to ascribe the phenomena to the general properties of the solution than to the special properties of colloids (**2**).

The data obtained in the study of this plant agree quite well with the experiments previously reported on colloidal and non-colloidial systems (**2**) wherein it was shown that the sign of the deviation depended upon the sum of the interacting forces within the solution and that the properties of the reference substances markedly affected the results. It is reasonable to conclude that in a natural solution consisting of water, organic acids, proteins and their intermediate products, one finds the same behavior regarding colligative properties, and the deviations therefrom, which may be expected in other complex solutions. The deviations observed are those characteristic of solutions in general.

In the case of Minhardi wheat the freezing-point depression and the deviations therefrom were most closely related to the change in amino-acid concentration. Doubtless other plants might respond differently. The fact should not be overlooked, however, that the principal factors involved in hardening may be other than this.

The deviations observed in these experiments seem satisfactorily explained by the properties of the solution due to charged ions, non-electrolytes, polarizable water molecules, and solute molecules of high dipole moment. Consideration of these facts suggests that any method based upon colligative measurements may be expected to give anomalous results when complex solutions are subjected to changes in concentration and that the deviations measure the sum of the effects of the various components upon water rather than the water bound by the colloid. The observations may be explained by assuming that when a non-electrolyte is added to sap from a hardened plant the ions and amino acids tend to "salt out"[3] the non-electrolyte and hence produce a positive deviation in the freezing point depression.

[3] "Salt-out" is a term applied to the decrease in solubility of proteins, gases, etc., by the addition of salts to the solution. "Salting in" refers to the increase in solubility. In a broader sense, these terms reflect the change in activity in components of a solution by the addition of another solute.

Likewise when a suitable electrolyte is added to the hardened sap the components having dipolar properties decrease the characteristic effect of the electrolyte upon the water; that is, the electrolyte is "salted in" and the magnitude of the freezing point depression or any other colligative measurement is less than expected. Thus any method involving great changes in concentration may lead to unexpected changes in the activity of water.

Data obtained by many workers by various methods indicate that some 0.4 and 0.5 grams of water per gram of colloid is associated with the colloid and has properties that differ from those of the solvent. The data of DEXTER (6, 7) and ROSA (31) indicate that wheat and cabbage plants enjoy definite protection at temperatures where the moisture content is well beyond the limit where bound water could be effective. While it is conceivable that some plants subjected to temperatures below the eutectic temperatures of the solutes of the sap might benefit by water retained in the liquid state through binding, perhaps the dominant factor involved in most cases is the unusual capacity of these plants to survive desiccation. It appears that the water in the sap of plants behaves just as it does in other aqueous solutions of similar complexity and may exhibit apparently abnormal behavior due to special properties of the components of the sap, especially crystalloidal components.

Summary

The bound water phenomenon has been studied in sap expressed from hardened and unhardened wheat plants of a typically hardy variety. The cryoscopic method was employed, using two types of reference substances.

The behavior of the biological solution has been interpreted on the basis of a similar study on artificial colloidal and non-colloidal solutions.

It appears that "bound" water in plant sap, as determined by colligative methods, is a reflection of all the components of the sap and of the reference substance where such is used. It is a hypothetical amount of water estimated to account for the difference in value of a property of water in complex solution and its value in simple solution.

Water in sap seems to have the normal properties of water found in any complex solution.

Changes in the thermodynamic activity of water in sap as plants harden may be accounted for most satisfactorily by the quantitative and qualitative changes in solutes as a result of physiological response to environment.

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THE RESPIRATORY METABOLISM OF McINTOSH APPLES DURING ONTOGENY, AS DETERMINED AT 22° C.

G. Krotkov

(WITH NINE FIGURES)

Introduction

Our knowledge of the respiratory metabolism of an apple fruit has been derived heretofore from examinations of continuous respiration records, and from the study of records so short, that they give nothing but the initial rates of respiration. The former are available only for apples in the later stages of their life (**1**, **3**); the latter provide information for apples in all stages of their ontogeny (**2**).

A respiration record of a starving fruit may be looked upon as an expression of the physiological state, in which this fruit was present at the beginning of examination. By comparing a number of such records, produced by fruits in various stages of their ontogeny, one can follow the changes in the physiological state of a fruit during its development. By extrapolating the tendencies observed during ontogeny to the time of commercial picking in the fall, one gets a picture of the physiological drifts in a fruit at that time.

Such a method of attack, based on the knowledge of the past history, can give valuable information which cannot be easily obtained from direct studies of a fruit already in storage. Since a search through the literature failed to reveal any complete sets of such records for any variety of apple, it was decided to obtain them. The McIntosh apple was selected as experimental material since it is one of our most important commercial varieties.

Materials and methods

The work reported below was carried out in 1939, following the preliminary tests of the preceding year. A full grown McIntosh tree was selected in one of the largest commercial orchards near Kingston, and all of the apples studied came from this tree. Apple blossoms began to drop their petals on June 1st, and this date was taken as the time of fruit setting.

Samples of apples were taken throughout their ontogeny, beginning from two weeks after setting, and continuing until the death in storage of all the apples picked later in the fall. During the summer and early in the fall, fruits were picked directly from the tree. On October 2, all the remaining apples were gathered, and a hamper of ungraded fruits was brought to the same constant temperature room in which all the samples were kept for study. They were spread on shelves in boxes and a stream

of air was drawn over them to remove the CO_2 produced. All of the samples taken after this date were selected from these apples. Throughout the ontogeny samples were taken for respiration studies and starch determinations.

Samples taken for respiration studies on June 14, 16, 27, and 29 consisted of six, six, five, and two apples, respectively. All subsequent samples were of one apple each. Previous to the taking of each sample, all available apples were examined, and only average looking fruits were selected. As soon as a sample was taken, notes were made on the color of the apple. The fruit was then brought to the laboratory, weighed, and enclosed in a glass respiration chamber,[1] which in turn was placed in a constant temperature room kept at $22 \pm 0.5°$ C. A stream of air drawn by a pump was passed over soda lime to free it from CO_2, over a weak solution of $Ba(OH)_2$ to bring it to a constant moisture content, over an apple in the respiration chamber and, finally, through a Pettenkoffer tube filled with 50 ml. of standard $Ba(OH)_2$. The Pettenkoffer tubes were changed daily, and the residual $Ba(OH)_2$ of each tube was titrated against standard HCl, using phenolphthalein as an indicator. The results have been expressed as milligrams of CO_2 produced per 100 grams of the initial apple fresh weight per hour.

Respiration of every sample was followed until the breakdown of the apple was apparent. With one exception, this appeared to have been caused by fungal infection spreading from the calyx. In only one case (sample 33), was it apparently due to some internal cause. One or two days previous to this apparent breakdown, there was a sudden and considerable increase in the CO_2 production, which apparently was produced while the internal organization of the apple was breaking down. In this work the respiration record was considered to have terminated immediately after the last low value, subsequent to which there was this increase in the CO_2 production, and one or two days later an apparent disintegration of the apple.

Samples of apples for the tests of their starch content with iodine were taken throughout the entire ontogeny of fruits. Each of these samples consisted of several apples, one of which was tested at once; the remainder were put in the same constant temperature room under conditions similar to those of the respiration samples. The starch content of these apples was determined later.

Results

Respiration records produced by different samples are shown in figures 1–4. Chronological number of each sample is given in the upper left-hand corner. At the bottom of each record there are letters, indicating the ground color of the apple at the time of its examination. Four colors were distin-

[1] Made by C. L. Muller, 6 Parton St., Red Lion Square, London, W. C. 1, England.

guished: green (G), yellowish-green (Y–G) greenish-yellow (G–Y) and yellow (Y).

Respiration of every apple from nos. 35 to 27 was represented essentially by the same gently declining line, which had been previously reported by several workers (**1, 3**) for the apples in storage. None of these apples were of pure green color, and all showed a transition from green to yellow. The most apparent point of difference among these records was that of duration: the earlier an apple was taken, the longer it lasted.

The respiration record of apple no. 25 was not of the same continuously declining type. It started with a low initial value, went up rapidly in the climacteric rise, after which it continuously sloped down, in the same man-

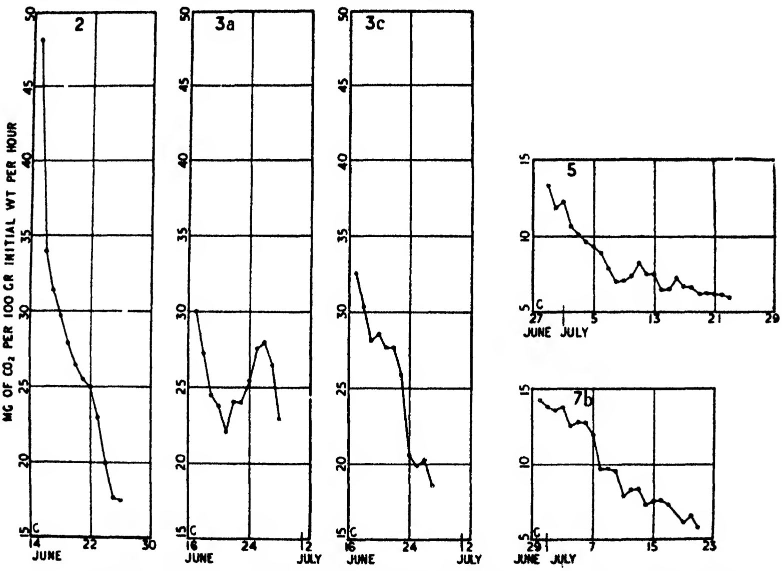

Fig. 1. Respiration records of apple fruits during starvation. The first date on the abscissa is the day of taking the sample.

ner, as did all the samples taken later. The climacteric rise for McIntosh apples was previously reported by Phillips (**4**).

Apple no. 23, which was taken six days earlier, not only displayed the same climacteric as did no. 25, but previous to this it exhibited a brief preclimacteric period of several days duration.

The record of apple no. 21, though essentially of the same type as that of no. 23, showed for the first time a new feature. Following a climacteric rise, it did not slope continuously down, as was the case in all of the apples taken later; after a brief decline it again rose slightly, and then resumed its steady fall. This temporary rise on the declining arm of the respiration record might be passed unobserved, if it were not for the fact that apples

picked earlier had this hump developed to a considerably greater degree. In the record of apple 13a this hump was even larger than the climacteric itself. This additional rise and fall has been provisionally called a "post-climacteric hump," since it occurred after the climacteric.

In the record of apple 11a this postclimacteric hump was obscure. In that of apple 9c it was again quite apparent, though developed to a considerably lesser extent than the climacteric. In addition, this hump shifted its place from that shortly after the climacteric, to the end of the record. A postclimacteric hump was absent from the records of all the samples taken earlier than was 9c.

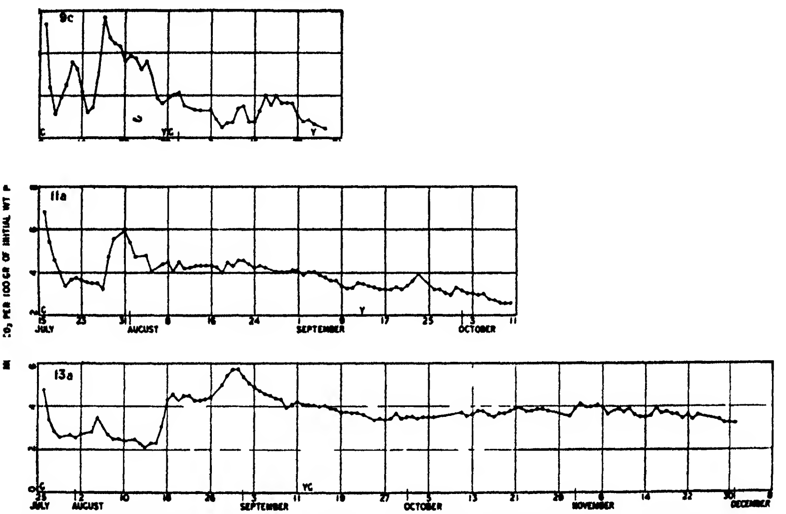

FIG. 2. Respiration records of apple fruits during starvation. The first date on the abscissa is the day of taking the sample.

While in the records of apples from 23 to 19 the preclimacteric period was represented by a straight line more or less parallel to abscissa, the first deviation from this type was produced by apple 17a. In this sample, the respiration record started with a high value, declined rapidly at first, and flattened out later. This deviation is still more pronounced in the record of apple 15a, and especially in that of 13a. In the last record there was an appearance of another possible feature of a preclimacteric period: an indication of a slight hump in its flat portion. This hump was displayed even more by apple 11a, and in apple 9c it was developed only slightly less at its peak, than was the climacteric itself. Since this hump was present in the preclimacteric period of a respiration record, it has been provisionally called a "preclimacteric hump."

All of the apples, whose respiration records are shown in figures 2 and 3, were initially green in their ground color, and all of them began to turn

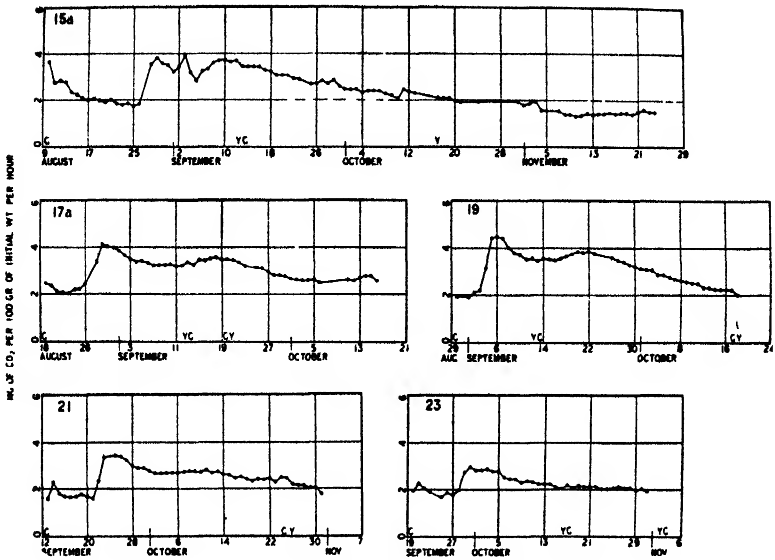

FIG. 3. Respiration records of apple fruits during starvation. The first date on the abscissa is the day of taking the sample.

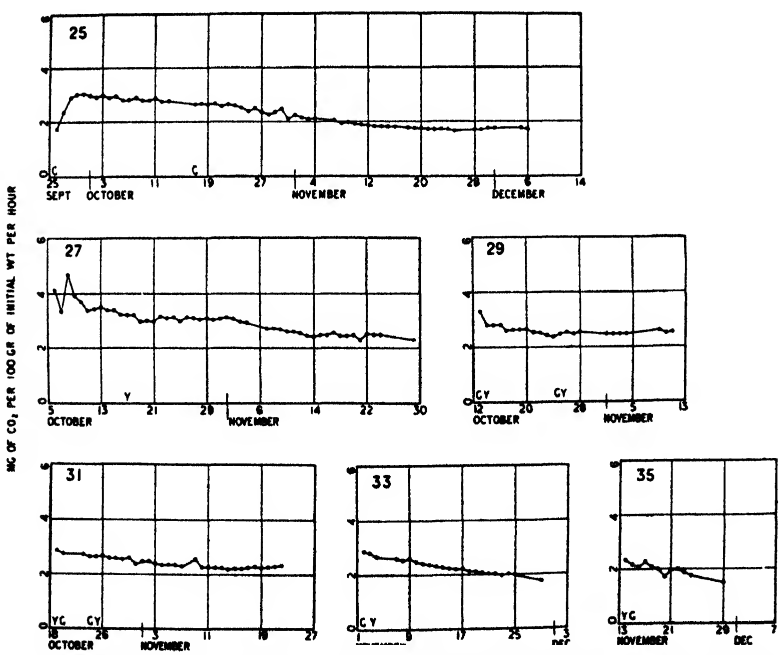

FIG. 4. Respiration records of apple fruits during starvation. The first date on the abscissa is the day of taking the sample.

yellow after the beginning of the climacteric. We can consider, therefore, that one of the signs of the onset of a climacteric, is this change in color from green to yellow.

Respiration records of samples of 2 to 7b were all essentially of the same declining type—falling more rapidly at first and with a tendency to flatten out later. On this type of record there might be present either one (sample 3a, 3c), or several (sample 7b) humps.

Tests for the presence of starch in apples revealed that no starch was present in apples picked on June 28th. On July 6th the first traces of starch were observed; from July 11th and until August 8th there was an abundance of starch in both cortex and pith. From August 15th there began a continuous decrease in the starch content until apples examined on October 12th either had no starch at all, or mere traces of it.

As the place for the storage of starch, the cortex of the receptacle (outside the ring of vascular bundles) was found to be far more important than the pith. In the earlier stages of ontogeny the appearance of starch in the pith lagged behind its appearance in cortex. In the later stages starch disappeared at first from the pith and only later from the cortex. Disappearance of starch from the cortex was found to take place in the three following stages:

(1) There was an appearance of clear areas without any starch in otherwise starch-containing tissue. This suggested at least a physiological heterogeneity of tissue; otherwise there should be a uniform decrease in the intensity of the blue color throughout the whole cortex.

(2) Starch-containing areas changed their color from very deep blue to lighter shades.

(3) There was a progressive disappearance of starch, starting from the center of an apple and spreading towards its periphery. The last traces of starch were always found under the skin.

Since exact quantitative data are lacking, no complete picture can be given now on the disappearance of starch from apples during starvation. The general trend of events is clear however. Starch is brought down from abundance to mere traces within two weeks, and when its initial amounts are low, this time may be shortened even more.

Analysis and interpretation of data

The initial respiration rates plotted against the time of taking samples are shown in figure 5. The graph obtained represents the changes in the respiratory potentiality of apples during their ontogeny, and is an approximation to the respiration of apples on a tree and in storage, when corrected to the same temperature of 22° C.

During the early part of its course this graph declines rapidly, while

later its fall is more gradual. Shortly before the time of picking of apples in October it goes up in the climacteric rise, following which there is a gentle decline until death in storage. This rapid decline of respiration during the first month of growth (June) corresponds to the period of cell multiplication. Absence of starch during this month is probably due to a heavy demand on sugars for the formation of new cells. When, in the following stage of cell enlargement, this demand subsides, then the excess of sugars is condensed into starch.

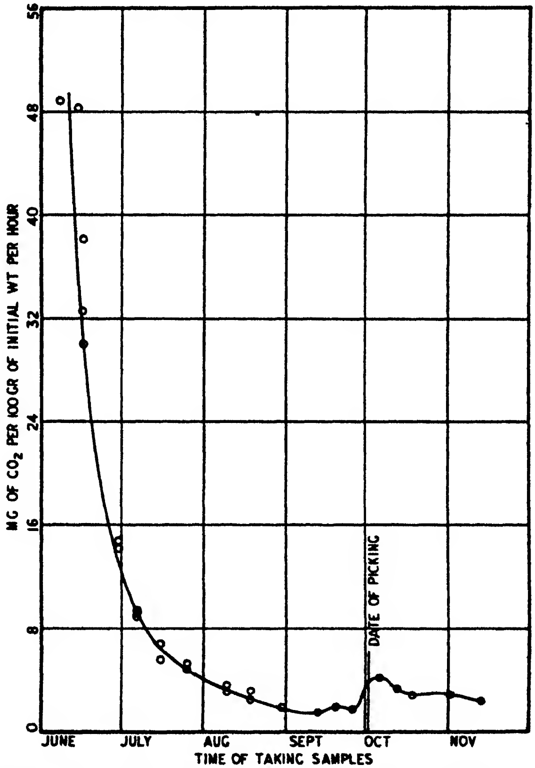

FIG. 5. Changes in the initial respiration rates of apple fruits during ontogeny.

Examination of figures 2 and 3 reveals that in each respiration record the minimal rates at the end of its preclimacteric and climacteric periods are usually not far apart. Figure 6 compares these two rates for the various samples of apples, and it is evident that these two are very close in each respiration record, and also that in different records they both vary in the same direction. Apples, taken after September 25, have no preclimacteric period; in such fruits only climacteric minimal rates are available.

Two explanations have been given as to the cause of the respiration rise in a climacteric. BLACKMAN (1) suggested that this is due to an increase

in the hydrolysis facilities of tissues, as result of which there is an increase in the concentration of the respiratory substrate. On the other hand, KIDD (2) considered it to be connected with some change in protoplasm itself.

The mutual proximity of the minimal preclimacteric and climacteric rates in each record can easily be explained as caused by changes in the concentration of the respiratory substrate. On this ground the observed decrease in the respiration during the preclimacteric period is due to the progessively decreasing concentrations of the respiratory substrate. When the amount of this substrate broken down just fails to yield the amount of energy necessary for the maintenance of the proper protoplasmic organization, then there is an appearance of either a new substrate, or, if still the same, at least from a new source. The increased concentrations of this new

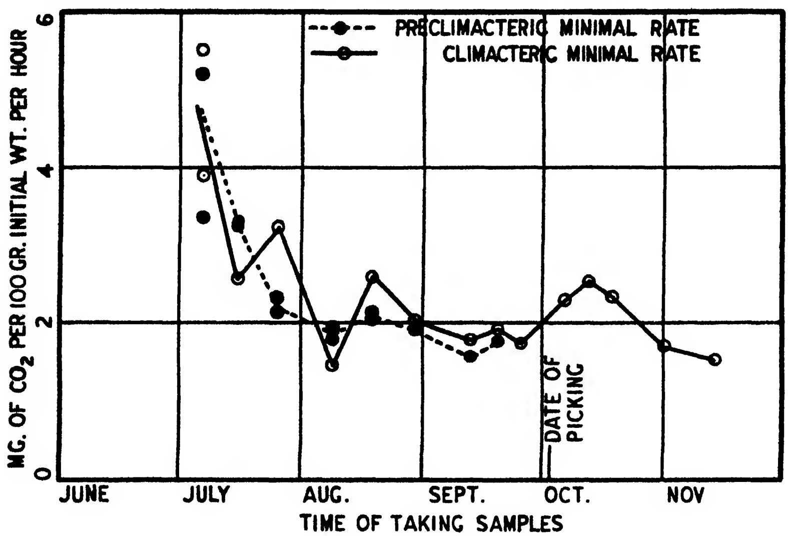

FIG. 6. Changes in the preclimacteric and climacteric minimal respiration rates of apple fruits during ontogeny.

substrate are causing an increase in the respiration rates resulting in the climacteric rise.

This new substrate, however, is eventually also brought down to such a low concentrataion that it fails to yield the necessary amount of energy. When this does happen, death occurs since there is no other source of the respiratory substrate to supply the deficiency. This mutual proximity of the minimal preclimacteric and climacteric respiration rates is due then to the fact that both represent the same thing; namely, the basal rate of metabolism for these apples under the described conditions. An increase of this rate in younger apples, and decrease in older, supports this view.

It was pointed out earlier in this paper that with the exception of one

sample, death appeared to have been caused by fungi. It is hardly a coincidence, that an apple always succumbed to a fungus at the time its climacteric respiratory rate dropped to the same low value, as it had been at the end of its preclimacteric period. A more probable explanation of this fact is

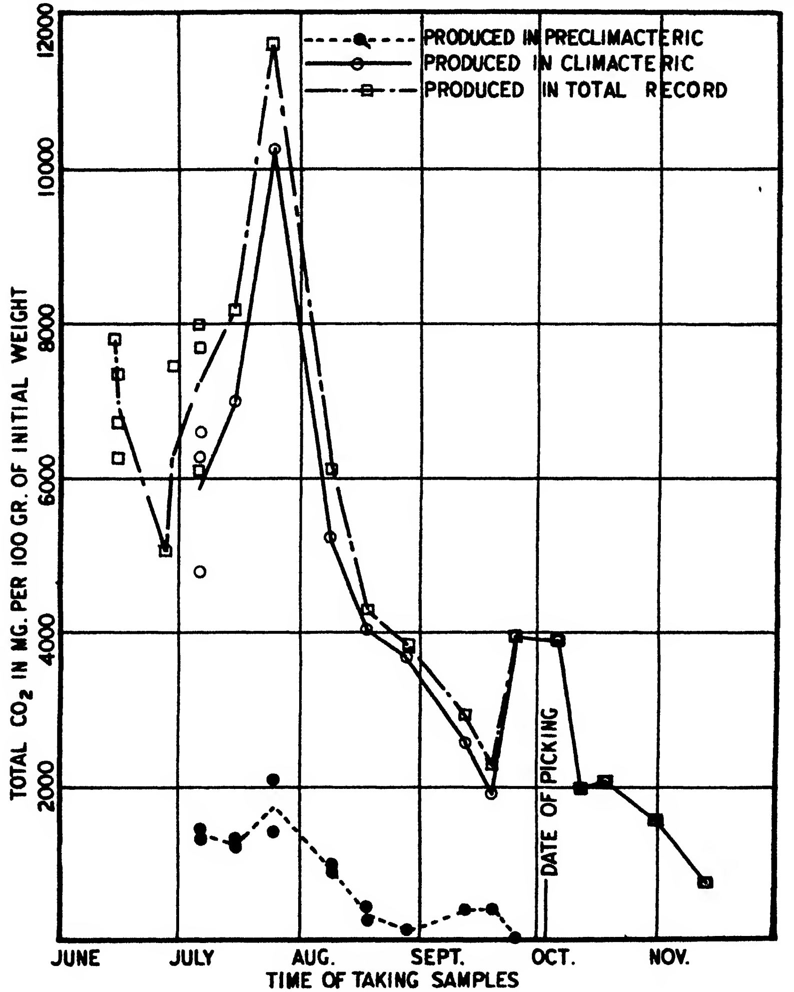

FIG. 7. Total CO_2 produced in starvation by apple fruits in various stages of ontogeny.

that a fungal infection did take place only when an apple has reached a definite physiological state, represented by a definite rate of respiration. According to the view outlined above, an apple succumbed to a fungal attack when its respiration fell below its basal metabolic rate, and the final disintegration of its protoplasmic organization already was beginning to take place.

In other words a fungal infection was one of the consequences of death, and not the cause of it.

The preclimacteric and postclimacteric humps are two other peaks which were observed on some respiration records. The preclimacteric hump was present in samples 13a, 11a, 9b, and, probably, in 3a. The reason why the hump produced by sample 3a is considered to be preclimacteric, lies in the absence of color changes. It was pointed out earlier in the paper, that a climacteric period is associated with the change of color from green to yellow. Since sample 3a remained green throughout its entire life, in the absence of any better methods for the identification of this period, the stand is taken that this hump is a preclimacteric and not a climacteric one.

Figure 7 gives for various samples the total CO_2 produced by the whole record, as well as CO_2 produced during its preclimacteric and climacteric periods. The graph for the total CO_2 production starts with a high value, and falls rapidly during the month of June.[2] It goes up from the beginning of July and reaches its peak by the end of the month; after this there is a continuous decline, rapid at first and slower later, interrupted only by a secondary peak at the time of the climacteric.

No separation of respiration records into the preclimacteric and climacteric periods can be seen in samples taken during the month of June. But when in later samples, such a separation becomes established, the climacteric period then contributes the bulk of CO_2. The graph for the CO_2 produced in the climacteric is at first closely parallel to that of the total CO_2; from the end of September, when the preclimacteric period disappears entirely, these two graphs merge into one.

The ground on which the hump exhibited by sample 3a is considered to be preclimacteric and not climacteric, has been given above. Other samples taken in June produced continually declining respiration records; humps, if present, were not followed by death; and, finally, apples did not show color changes from green to yellow.

On all these grounds respiration records of June samples have to be considered as represented by the preclimacteric periods only. On the basis of this conclusion figure 8 gives the total CO_2 of both preclimacteric and climacteric periods of respiration, produced by apples in various stages of their ontogeny. In June, when apples are present in the stage of cell multiplication, all their CO_2 is produced in the preclimacteric period. The onset of cell enlargement brings a steady decline of the preclimacteric, interrupted only by two secondary peaks, and by the end of September this

[2] The two last values of this month, while placed on this graph correctly chronologically, ontogenetically should be plotted earlier. Samples 7a and 7b, taken on June 29th, both were characterized by higher initial respiration rates, than sample 5 taken on June 27th.

period disappears completely. On the other hand the climacteric period does not appear until apples have entered the stage of cell enlargement. But once established it gains rapidly in magnitude, reaching a peak a few weeks later, and then declines until the complete extinction of the pre-

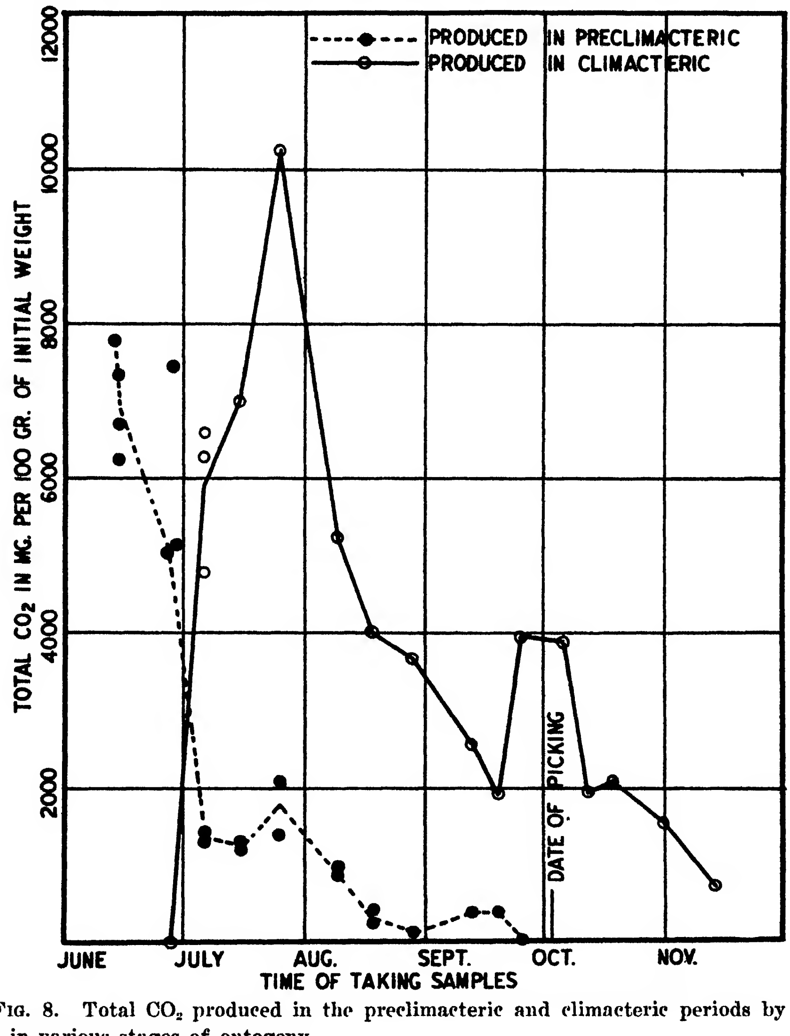

FIG. 8. Total CO_2 produced in the preclimacteric and climacteric periods by apple fruits in various stages of ontogeny.

climacteric period. From this time on the climacteric is the only period represented in respiration.

Figure 9 presents, for the various samples, the length in days of total respiration records, and the respective preclimacteric and climacteric periods. A careful examination of this figure, and its comparison with

figure 7, reveals a considerable similarity between the two. The most apparent difference is the short life of apples picked in June, in spite of the large amounts of CO_2 produced by them. But from the beginning of July onward, the total life of an apple, and the duration of its preclimacteric and climacteric periods are all directly proportional to the total amounts

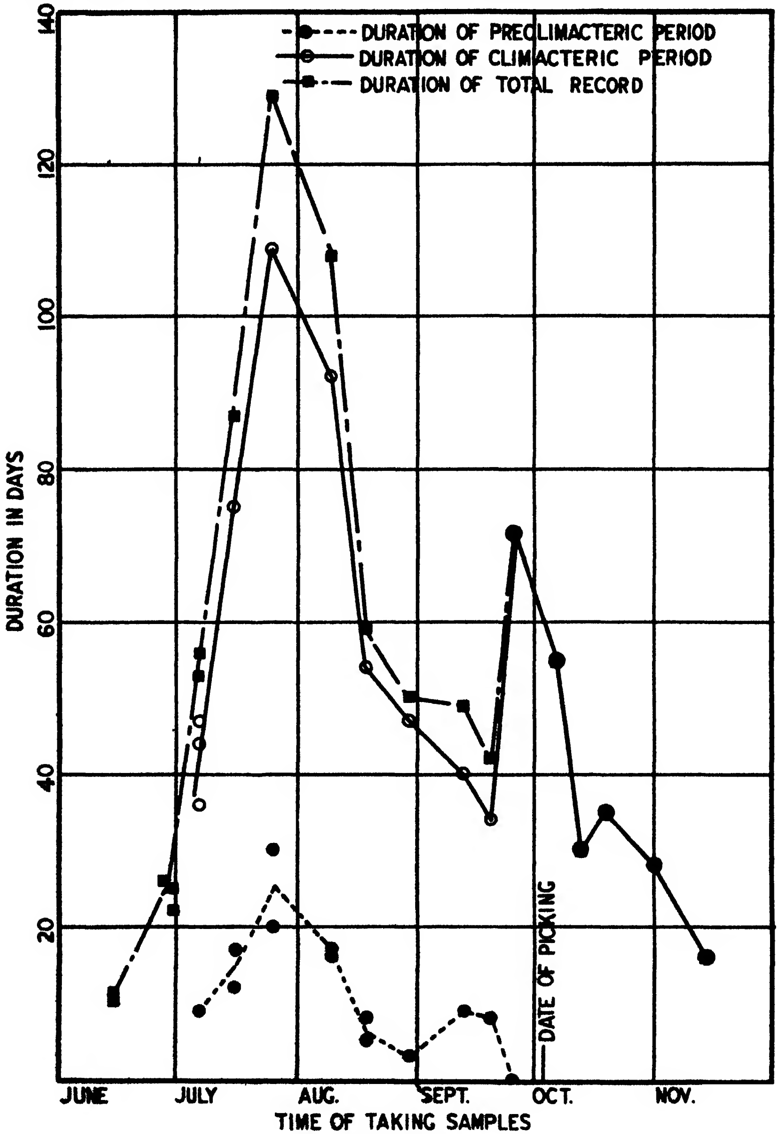

FIG. 9. Duration in days of the total respiration records, and of their respective periods, as observed in apple fruits present in various stages of ontogeny.

of CO_2 produced in them. Since during this time CO_2 is produced either mainly or exclusively in the climacteric period, the duration of life depends now on the total amounts of the substrate for this period present in an apple. The larger these amounts, the longer an apple will last. The practical importance of more work for the elucidation of the nature of this substrate and of the conditions favoring its accumulation is obvious.

The work reported above was actually carried out using as experimental materials, not only McIntosh but Northern Spy apples as well. The data obtained for both apples, while different quantitatively, were so alike qualitatively, that in some cases data for the Spy apples helped in the interpretation of those for the McIntosh. The only important point of difference between the two varieties is the greater sensitivity of Spy apples to higher temperatures in later stages of their ontogeny. Thus while early Spy samples produced respiration records strikingly similar to those of McIntosh, samples studied in October began to disintegrate before their climacteric rate reached a value comparable to their minimal preclimacteric value. Duration of life in such Spies was considerably less than in McIntosh apples.

Summary

1. A number of continuous respiration records of McIntosh apples is presented. These were produced by samples of apples taken throughout the whole ontogeny of the fruits, and stored at 22° C.

2. A complete respiration record of a fruit is found to consist of a preclimacteric and a climacteric period. The relative importance of these two periods in various stages of fruit ontogeny is described. It is shown that the earliest records are represented by the preclimacteric and the latest by the climacteric periods only.

3. A tentative explanation is given of changes in a complete respiration record, based on the assumption that these are due to variations in the concentration of the respiratory substrate.

4. From the data presented it is concluded that a fungal infection did take place only after an apple had reached a definite physiological stage of its starvation, and when disintegration of its protoplasmic organization already was taking place. Fungal infection, consequently, was one of the consequences of death, not the cause of it.

5. The life in days is given for the apples in various stages of their ontogeny. From the beginning of July and onward, a direct proportionality is observed between the duration of life, and the total amounts of the CO_2 produced by apples in the climacteric period of their respiration.

6. The value of the reported work is considered to be two-fold: first, it gives a general idea of the respiratory metabolism of McIntosh apples during their ontogeny; second, it may serve as a kind of time table, so that if in

the future it is decided to investigate in detail any particular stage of metabolism, one can choose for this purpose the best suited age of apples, and one knows how far apart samples for analysis should be taken.

The author wishes to express his thanks to Mr. L. Day for the technical help in carrying out these experiments, and to Mr. P. H. LaBrosse who kindly provided all of the experimental material from his orchard. Part of the expense of this research was defrayed by a grant from the Science Research Committee of Queen's University.

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THE EFFECT ON BARLEY SEEDLINGS OF SOME INTER-RELATIONS OF CATIONS AND ANIONS IN A THREE-SALT NUTRIENT SOLUTION

WENDELL R. MULLISON

(WITH TWO FIGURES)

Many investigations have been made of the nutrition of plants grown in sand and water culture, and more particularly of the results of the deficiency of one or more specified ions in an otherwise complete nutrient solution. In almost every case, the deficient element was replaced in the nutrient solution by another one.

Recently a method was described by HAMNER (4)[1] whereby the anions or the cations of a three-salt nutrient solution could be varied and yet have the rest of the nutrient solution almost constant without the addition of other elements than the original ones. Thus, calcium, magnesium, and potassium could be varied while the nitrates, sulphates, and phosphates remained nearly constant, and *vice versa*. By this method, involving the use of two triangle systems, the concentrations of only three ions, cations or anions as desired, are varied whereas in the ordinary triangles both the anions and cations are varied, thus resulting in the variations of all six ions at the same time (7).

This method was first used in studying growth responses of a legume, and since this type of plant often has a different physiological response from other plants, the following experiments were carried out with barley, a member of the grass family.

Barley seeds selected for uniformity were planted in quartz sand in glazed 4 × 8-inch self-draining crocks. Ten seeds were planted in each crock and three crocks were used for each point of the triangle. Plants were watered with distilled water until the first leaf was about 5 cm. tall, after which they were watered on alternate days with nutrient solution and were flushed out each week with distilled water.

All experiments were repeated twice later in the summer, so that a total of about four thousand plants was used. The investigations were begun in June and continued through September. Plants were grown under ordinary greenhouse conditions of light and humidity.

Two nutrient triangles were used. In the first triangle, three stock solutions, N, S, and P were prepared. Solution N consisted of 0.0045 molar KNO_3, 0.0045 molar $Mg(NO_3)_2$, and 0.006 molar $Ca(NO_3)_2$. Solution S consisted of 0.0045 molar K_2SO_4, 0.0045 molar $MgSO_4$, and 0.006

[1] Somewhat similar attacks upon this problem have been made by BECKENBACH, WADLEIGH, and SHIVE (1), and WADLEIGH (8).

molar $CaSO_4$. Solution P consisted of 0.006 molar $Ca(H_2PO_4)_2$, 0.0045 molar KH_2PO_4, and 0.0045 molar $Mg(H_2PO_4)_2$. The latter compound rather than $MgHPO_4$ was used because of its greater solubility and because its use allows the anions to be kept more nearly constant. The $CaSO_4$, instead of being added as such, was made by adding equivalent amounts of finely powdered $CaCO_3$ and H_2SO_4. This was done because of the slowness with which $CaSO_4$ dissolves. By these two modifications, it is possible to have all components go quickly into solution and to avoid the formation of a fine sludge in certain of the stock solutions which often occurs if the original method is followed.

By varying the amounts used of each of the solutions N, S, and P, the anions could be varied, while the cations remained practically constant.

In the second triangle there were three stock solutions: M, K, and C. Solution M consisted of 0.0045 molar $Mg(NO_3)_2$, 0.0045 molar $MgSO_4$, and 0.0045 molar $Mg(H_2PO_4)_2$. Solution K consisted of 0.0045 molar KNO_3, 0.0045 molar K_2SO_4, and 0.0045 molar KH_2PO_4. Solution C consisted of 0.006 molar $Ca(NO_3)_2$, 0.006 molar $Ca(H_2PO_4)_2$, and 0.006 molar $CaSO_4$, the latter being added by the same method as described above for the first triangle. By varying the amounts of each one of the nutrient solutions M, K, and C the cations can be varied at will while the anions remain practically constant.

In the first triangle, plants at one corner were watered only with solution N, at a second corner with S, and at the third only with P. Similarly, in the second triangle, the corner plants were watered only with solution M, or K, or C. All intermediate points of each triangle were watered with solutions made by mixing together various amounts of the three stock solutions, so that each point on the triangle varied by $\frac{1}{6}$ from every other point, thus making a total of 28 different nutrient solutions in each triangle (see figs. 1 and 2).

In addition to the plants in the triangle, a group of 50 was set aside as a control and was fed with a three-salt nutrient solution composed of 0.006 molar $Ca(NO_3)_2$, 0.0045 molar KH_2PO_4, and 0.0045 molar $MgSO_4$.

All plants were harvested when twenty days old. Heights of plants and dry weights of roots and tops are recorded in figures 1 and 2. Since the light was much poorer during August than during July, the plants grown later in the season did not synthesize as much dry matter and were somewhat slower to respond to the various treatments than were those grown earlier, but the same relative results were obtained.

Results and discussion

TRIANGLE I. NO_3, SO_4, PO_4 VARIED; Mg, K, Ca CONSTANT

Initial deficiency symptoms in this triangle appeared within three or

four days after the first application of nutrients. In that series along the side of the triangle in which there was a complete absence of NO_3, and varying amounts of SO_4 and PO_4, the leaf tips began to show a slight yellowing, followed shortly by a progressive death of the leaf from tip toward base. This necrosis occurred where the SO_4 was decreased to $\frac{2}{6}$ or less than $\frac{2}{6}$ of its maximum value, becoming more severe as the SO_4 decreased

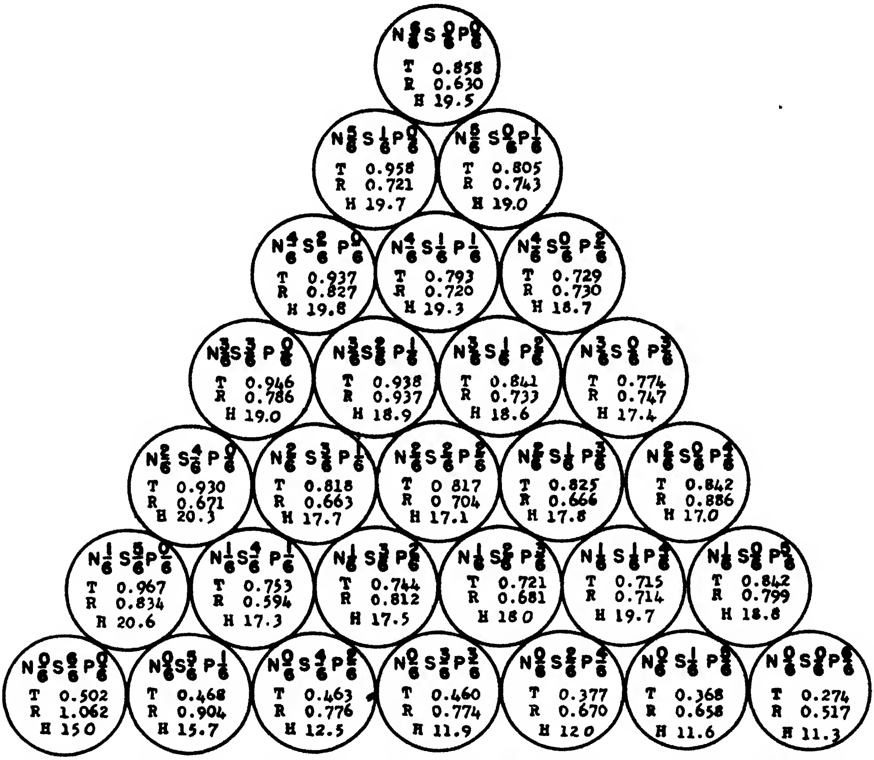

FIG. 1. Triangle I. Each point of the triangle was watered with the fractions of solutions N, S and P indicated in each circle. Solution N contains various nitrates; S, sulphates; and P, phosphates. T and R indicate dry weights in grams of tops and roots, respectively. H indicates average heights in cm. of plants in each treatment.

Data for the controls:

Tops	= 0.877	grams
Roots	= 0.764	grams
Height	= 18	cm.

and the PO_4 increased. The plants given the solution in which the PO_4 was increased to its maximum and the SO_4 omitted were almost entirely dead by the time of harvest. On the other hand, if the SO_4 was raised to $\frac{5}{6}$ of its maximum and the PO_4 reduced to $\frac{1}{6}$, the plants were only slightly stunted and a little yellowed because of the lack of NO_3. Whether this decrease in

toxicity with the increasing amount of SO_4 is caused by the actual presence of larger amounts of SO_4 or merely by the decreased amount of PO_4 is not known, since as the SO_4 increases the PO_4 decreases according to the set-up of the triangle.

In that series watered with solutions containing no SO_4 but having varying relative amounts of NO_3 and PO_4, the same toxic symptoms appeared when PO_4 was relatively high in amount and the NO_3 low, although these plants were markedly better than those given the SO_4–PO_4 complex in the absence of NO_3. NO_3 was highly effective in preventing the toxic effect ot excessive PO_4, even a small amount of NO_3 preventing the toxic effect of a large amount of PO_4.

That series of plants supplied with a solution lacking PO_4 and with varying relative amounts of NO_3 and SO_4 were uniformly good, the plants at the time of harvest being still too young for the PO_4 deficiency to have been manifested. All this points definitely to a pronounced PO_4 toxicity when NO_3 is absent, or present in only a low concentration. This has been previously shown by HAMNER (4) in his work on bean seedlings. MOORE (6) also found evidence of PO_4 toxicity in peanut seedlings.

The results of the treatments in the central regions of the triangle showed such slight variations as not to be significant, those with relatively low N being almost as vigorous as those with much N.

That solution containing only NO_3 and no SO_4 or PO_4 produced plants comparable to those receiving balanced solutions. This is probably because there were enough materials in the seeds to take care of the deficiencies in the solutions until the time of harvest. The solution containing only SO_4 as the cation produced plants which, although stunted and a little lighter green because of the lack of NO_3, were nevertheless in good condition. This was in marked contrast to those plants receiving PO_4 as the only cation. The latter were very poor. This again indicates the toxicity of PO_4 in the absence of NO_3.

In this triangle the roots were but slightly affected by the different nutritional treatments. Their size and weights were all remarkably similar, except for that series given the solution entirely lacking NO_3 and having varying amounts of SO_4 and PO_4. Here again, as in the case of the tops, there was a definite decrease in weight as the PO_4 increased and the SO_4 decreased.

TRIANGLE II. Mg, K, Ca VARIED; NO_3, SO_4, PO_4 CONSTANT

Here the first and the most severe symptoms to appear were those of K deficiency. In fact, these manifestations appeared in 2 to 3 days, even before the PO_4 toxicity or the NO_3 deficiency in triangle I. The first apparent reaction to K deficiency was a stunting of the plant, which was much

more marked than the stunting brought about in a comparable period of time by lack of N. This stunting was followed rapidly by a yellowing and subsequent browning of the leaf starting at the tip and progressing towards the base. This occurred in the entire series of plants on that side of the triangle lacking K and having varying amounts of Ca and Mg. Here the plants were all uniformly very poor, there being apparently no Ca-Mg relationship affecting this die-back. Whether high in Ca and low in Mg or

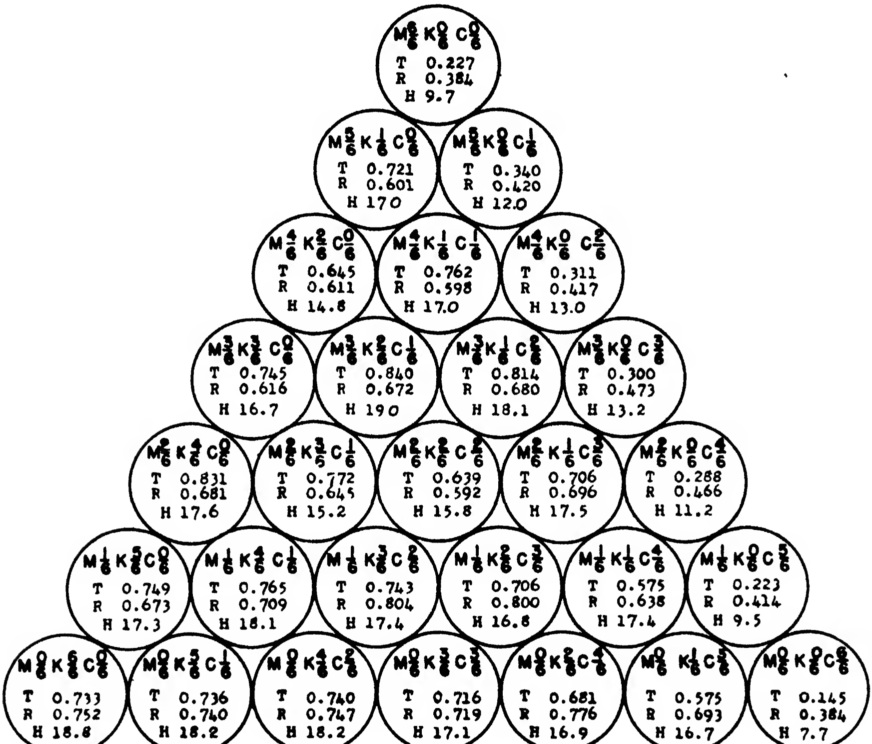

FIG. 2. Triangle II. Each point of the triangle was watered with the fractions of solutions M, K, and C indicated in each circle. Solution M contains Mg salts; K, potassium salts; C, calcium salts. T and R indicate dry weights in grams of tops and roots, respectively. H indicates average heights in cm. of plants in each treatment.

vice versa, the K deficiency symptoms appeared at about the same time and progressed at about the same rate. The reaction was so severe that in some cases more than ⅓ of the plant tops were dead at the time of harvest. Those plants given the solution in which Mg was the only cation, however, reacted somewhat differently. Instead of yellowing they became a creamy white, and the tips shriveled more slowly than did the others, so that the basal parts remained white and without withering for a long time; finally

only ½-inch of the extreme basal parts of the leaves remained a healthy green color. This reaction is undoubtedly caused by the rôle which Mg plays in the formation of the chlorophyll molecule.

In the series on the side of the triangle given the solutions containing no Ca and varying amounts of Mg and K, the leaves were not seriously affected as they were in the series lacking K. Here where the amount of Mg was high as compared with K, the growing points were killed. This occurred in all cases where the Mg was more than ½ of its possible maximum. Even where it was ½, there were many individual cases with dead growing points. Where the Mg was decreased to ⅓ its maximum value, however, and K increased to ⅔, the growing points were usually unaffected; any further decrease in the Mg left the growing points healthy. Thus, in the absence of both Ca and Mg in the nutrient solution the growing points remained healthy. This points definitely to a toxic effect of Mg, rather than to a Ca deficiency in these seedlings. GAUCH (3) in his work on bean seedlings points out the extreme toxicity of Mg in the absence of Ca. It is not known whether variations in the concentration of K may affect this Mg toxicity manifested in the absence of Ca, since in accordance with the setup of this triangle as the K concentration increases, the Mg concentration is proportionately decreased. Therefore, this decrease of injury with the increasing K may be caused simply by the accompanying reduction in the amount of Mg.

The plants on the side of the triangle lacking Mg were fairly good. They were best at the end where the concentration of K was highest, and decreased in weight as the K decreased and the Ca increased.

Of the three cations present in the nutrient solution, the K was the most important one in maintaining the plants in a vigorous state. Deficiency of K was the first deficiency to appear and plants suffering from it were the poorest of all deficient plants at the time of harvest.

Since Ca and K, particularly the latter, are known to be associated with root growth, it might be expected that the roots would be markedly affected by the different nutritional treatments in this triangle. They were actually affected to such a degree that in some cases they were less than ½ the normal size and weight. The most pronounced effect was in that series lacking K. Although in this series the amount of Ca varied inversely with the amount of Mg in the nutrient solutions, the roots were in all cases rather uniformly stunted. The roots, although dwarfed, were apparently healthy.

Considering all plants in both triangles, it may be said that in general those given the solutions in which the K, Ca, and Mg were kept constant and the NO_3, SO_4, and PO_4 were varied did better than the plants in the other triangle. The most vigorous plants, in either triangle, however, compared very favorably with those in the other. Certain combinations of nutrients

in the triangles produced plants that are comparable with those given the three-salt nutrient solution. The former are the equals of the latter in every way. Data for the controls are found in the legend for figure 1.

GREGORY (**2**) based his mathematical approach to this problem upon the false philosophical assumption that the effects of the ions involved are additive and did not allow for interaction among them. He recognized this fault but used the assumption for the purpose of simplification. Nevertheless, several of his conclusions are in accord with experimental evidence presented in this paper, and it is still more interesting when one considers that his data came from JOHNSTON (**5**), who had designed his experiment for an entirely different purpose. The author disagrees with GREGORY in one important point. The latter finds Ca to be the most important cation and K the least while the present work indicates K to be more important than Ca.

In arriving at these conclusions for the work reported here, it must be remembered that the experiments were with seedlings and these results might be different if the plants were grown to maturity.

Summary

1. A study was made of the effect on barley seedlings of the interrelations of the cations and the anions present in a three-salt nutrient solution.

2. The plants of the triangle in which the anions were varied were as a whole better than plants of the triangle in which the cations varied.

3. The variations in the nutrient solutions had their greatest effect upon the top growth of the plants. The root growth was much more uniform except in the absence of K which caused a marked decrease in size and weight of roots.

4. In the absence of nitrates, or when a high phosphate-low nitrate relationship existed a marked phosphorous toxicity became apparent.

5. Potassium deficiency symptoms appeared extremely early, before signs of deficiency or toxicity of any other element. A severe die back of the leaves of these seedlings resulted.

6. A high concentration of Mg in conjunction with an absence of Ca in the nutrient solution resulted in the death of the terminal meristem, and caused some stunting and chlorosis.

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MOISTURE DISTRIBUTION IN SOIL IN CONTAINERS

A. H. HENDRICKSON AND F. J. VEIHMEYER

(WITH THREE FIGURES)

In many problems dealing with the use of water by plants, containers of various sizes are adopted, in which the system is sealed against loss of moisture by evaporation from the surface, and against loss by drainage. These containers are usually weighed at intervals, and water added in measured quantities to bring about the moisture conditions desired by the investigator. Generally, these experiments contain several series of containers that are supposed to be moistened uniformly, and kept at moisture contents less than that of the field capacity of the soil used. A report was made on the distribution of moisture in soil following an irrigation in various field experiments (**1, 3, 5, 6**), in which it was shown that water moves but slowly from soil at field capacity to adjoining dry soil, and that, in the absence of roots, the line of demarcation between the wet and the dry soil does not change during relatively long periods of time. In another paper (**2**) evidence was presented which showed that the movement of moisture from a wet to a dry soil in containers, takes place in the same way that it does under field conditions. The lack of uniform distribution of moisture by autoirrigators was shown by the accumulation of roots immediately around and below the porous cones. A device for automatically bringing the moisture in a soil in a container up to the field capacity is described by JOHNSTON and ATKINS (**4**).

The following results show some of the soil-moisture conditions obtained in experiments with sunflower plants in containers, when the soil was wetted to the field capacity, and when attempts were made to moisten soil at the permanent wilting percentage, to a moisture content less than that of the field capacity. Three soils, a sand, a loam, and a clay adobe, were used. The containers were all brought to a uniform weight for convenience in handling, and a known weight (about 600 grams) of oven dry soil was placed in each. The soil was moistened to its field capacity and dwarf sunflowers planted. When the sunflower plants had reached a height of about 12 cm. and each had 3 pairs of full sized leaves, they were allowed to wilt. The wilting percentages obtained agreed with those obtained in previous trials with these soils. After wilting, various amounts of water were added to the surface of the soil. The plants were then cut off and the container sealed until the following day when the cans were cut longitudinally with a hack saw, and photographed. Soil samples of the top and bottom portions were taken for the determination of the moisture content. The moisture equiva-

lents, which agreed closely with the field capacities of these soils, were obtained from the stock supply by centrifuging in the usual way.

The penetration of water was not uniform, and there was a tendency for the water to move down along the sides of the can. This tendency was more pronounced with the loam and clay adobe soils than with the sand. In addition to the cracks around the sides of the container, the loam and clay adobe soils had other cracks on the surface that permitted deeper penetration of water in some places than in others. Difficulty was experienced in taking the samples to make sure that some dry soil was not included in the moist sample, particularly when the samples were being taken near the margin of penetration. The samples from the bottom of the cans frequently showed evidence of moisture that had moved down through the cracks.

The results obtained are given in table I.

TABLE I

MOISTURE DISTRIBUTION IN SOILS IN CONTAINERS IN WHICH THE SOILS WERE FIRST REDUCED TO THE PERMANENT WILTING PERCENTAGE AND THEN WERE WATERED

NUMBER	KIND OF SOIL	MOISTURE EQUIVALENT	PERMANENT WILTING PERCENTAGE	CALCULATED MOISTURE CONTENT BY WEIGHT AFTER WATERING	ACTUAL MOISTURE CONTENT	
					WETTED PORTION	DRY PORTION
		%	%	%	%	%
1	Fresno sandy loam	9.3	2.2	3.8	8.3	3.0
2	"	9.3	2.2	4.4	8.1	2.6
3	"	9.3	2.2	5.4	10.4	3.1
4	Dublin adobe	26.0	13.6	22.9	24.6	16.3
5	"	26.0	13.6	22.9	28.3	16.5
6	"	28.1	14.6	20.8	22.3	16.9
7	"	28.1	14.6	18.1	19.4	17.6
8	"	28.1	14.6	19.6	24.1	15.1
9	"	27.0	14.7	17.3	22.4	14.9
10	"	27.0	14.7	17.8	23.3	14.3
11	"	22.6	12.1	22.7	25.7	20.4
12	Yolo loam	23.5	11.9	18.0	20.6	11.9
13	"	24.0	11.9	15.0	19.4	11.3
14	"	24.0	11.9	16.1	25.5	12.2
15	"	24.0	11.9	23.5	22.3	20.9
16	"	24.0	11.9	24.2	22.3	21.2
17	"	23.5	11.8		12.1	12.1
18	"	23.5	11.8		11.8	11.4

In the column headed "Calculated moisture content by weight after watering," are given the percentages of moisture in the soil, if the amounts of water had been uniformly distributed. Some of these would approximately correspond to the terms "high," "medium," and "low" moisture

contents frequently found in the literature on this subject. The true moisture contents found are given in the last two columns. The soil-moisture percentages in the table are on an oven-dry weight basis.

In the case of the three samples of Fresno sandy loam, water was added to the surface, to give, if the water had been uniformly distributed, moisture contents between the moisture equivalent and permanent wilting percentage. The results show, however, that the moisture contents of the upper portion of the soil were near the moisture equivalent, and those of the lower portion were near the permanent wilting percentage. Figure 1 shows the result of

FIG. 1. Depth of penetration in a Fresno sandy loam soil when moisture was added to bring the average soil-moisture content by weight from the permanent wilting percentage (2.2 per cent.) up to 5.4 per cent.

attempting to bring sample 3 to a medium moisture content.

Similar results were secured with the Dublin clay adobe. Because of the large cracks, it was difficult to secure samples near the bottoms of the containers that did not show evidence of having been moistened. Samples 4, 5, and 6 showed that some water had penetrated through cracks to the bottoms of the cans. In some cases the movement of water down through the cracks was so rapid that portions of the soil in the upper portion of the

container remained dry. The unavoidable inclusions of this dry soil in the samples gave average moisture contents below that of the moisture equivalent because of the mixture of wet and dry portions of the soil. Sample 7 was severely cracked and the average moisture contents agreed fairly well with the calculated content, although wet and dry areas could readily be distinguished on the surface of the soil when the can was cut. Samples 8, 9, and 10 show fairly close agreement between the permanent wilting percentage and the moisture content of the soil in the bottom portion of the can

FIG. 2. Typical example of distribution of moisture in Dublin clay adobe soil, when water was added to bring the average soil-moisture content by weight from the permanent wilting percentage (14.6 per cent.) up to 19.6 per cent.

indicating that no moisture had penetrated to the bottom. Sample 11 was brought up to the moisture equivalent, but it is possible that the soil samples were taken before the moisture in excess of the moisture equivalent in the top portion had time to drain out, because the top layer was slightly above the moisture equivalent, while the bottom layer was slightly below. The uneven distribution of moisture that frequently occurs with this type of soil, unless enough water is added to bring the whole soil mass to the moisture equivalent is shown in figure 2.

Trials with the Yolo soil yielded results that were similar to those obtained with the other two. Samples 12, 13, and 14 showed moisture contents in the upper portion in excess of the desired average, while the bottom layer remained at the permanent wilting percentage. The penetration of moisture in sample 12 is shown in figure 3. In sample 12 approximately half

FIG. 3. Typical example of distribution in a Yolo loam soil when water was added to bring the average soil moisture content by weight from the permanent wilting percentage (11.9 per cent.) up to 18.0 per cent.

of the water required to bring the moisture content of the entire soil mass up to the moisture equivalent was added. The result was that only about half of the soil was wetted. Water penetration into the soil was greater along the sides of the can than it was directly under the plant. Samples 15 and 16 were brought up to the moisture equivalent and the actual moisture contents of top and bottom portions show fair agreement with this value. The agreement between the permanent wilting percentages and the soil-moisture contents of the top and bottom portions of two cans in which the plants were simply allowed to wilt is shown in samples 17 and 18.

The actual moisture contents of the soil in the top portions of the cans

were lower than the moisture equivalent in a number of instances. These differences may be accounted for, in part, at least, by the fact that cutting the cans was a slow process and it is probable that some moisture was lost from the exposed surfaces of the soil. Furthermore, it is probable that because of the uneven penetration of water, some dry soil was inadvertently included in the dry samples, particularly when the samples were taken close to the demarcation zone between the wetter and drier portions.

The results presented show that, when a uniform distribution of moisture is desired for plants growing in a container it is necessary to add sufficient water to wet the entire soil mass to the moisture equivalent. Otherwise, when less than this amount is added, only the top layer is moistened to the moisture equivalent while the soil at the bottom remains dry, if no cracks are present. Plants growing in soil held under "medium" soil moisture conditions are not under uniform conditions but simply function with part of their roots in the moist top layer, and part in the dry layer beneath. Movement by capillarity is so slow that the plant exhausts the available moisture in the top layer before any appreciable movement can take place.

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EFFECTS OF INJURIES CAUSED BY THE CICADA, *MAGICICADA SEPTENDECIM*, ON THE LATER GROWTH OF TREES

GREGORY THOENNES

(WITH ONE FIGURE)

It is a well-known fact that branches attacked by cicadas often die beyond the point of injury. The effects of injuries too slight to cause the death of the branch, however, have been rarely investigated. WILSON (2) reported damage to asparagus. WYMORE (3) also reported that prune and apricot trees were affected, and that the insect was apparently a carrier of disease to asparagus. The damage in these cases, however, was mainly the result of the feeding of the nymphs on the foliage or roots. The damage resulting to the trees in consequence of the egg-laying activities of the adults seemingly has been overlooked. ANDREWS (1), while pointing out that these injuries may hamper the trees more than seems to be the case at first sight, seems to think, however, that the biological curiosity aroused by the cicada more than compensates for the harm done.

Procedure

In 1934 the emergence of a swarm of cicadas in eastern Missouri provided the opportunity to study the effects of such injuries over a long period of time. During the six year period, 1934 to 1940, measurements of the yearly increase in growth of normal and damaged branches were taken. Both orchard and non-orchard varieties were included in the study. The orchard varieties studied were the apple and peach. The non-orchard types included the white oak, buckeye, and hard maple. In all cases the normal and injured branches measured were taken from the same tree and, as nearly as possible, in the same relative position on the tree. Branches showing injuries attributable to any other causes were carefully excluded. Thus, variations in growth owing to such conditions as sunlight, moisture, etc., could be eliminated. Measurements were taken of as many branches as possible from at least twenty trees of each type, and the data here presented (table I) represents, in every case, the average of these figures. The yearly percentage of growth was calculated on the basis of the total growth of the normal branches.

Results

Of the orchard varieties, the apple trees seem to have suffered the most harm. The cicadas attacked them in great numbers, and it is difficult to find a tree, standing at that time, whose branches do not bear scars marking the egg-laying activities of these insects. Although the wounds have healed

TABLE I

COMPARISON OF GROWTH OF TRFES INJURED BY CICADAS TO THE NORMAL GROWTH

YEAR	APPLE				PEACH				BUCKEYE				HARD MAPLE				WHITE OAK			
	INJURED		NORMAL		INJURED		NORMAL		INJURED		NORMAL		INJURED		NORMAL		INJURED		NORMAL	
	CM.	%	CM.	%	CM.	%	CM.	%	CM.	%	CM.	%	CM.	%	CM.	%	CM.	%	CM.	%
1940	4.8	2.2	13.7	6.2	35.7	11.6	35.1	11.3	0.8	1.9	2.5	6.1	1.4	1.5	3.2	3.5	7.4	7.4	8.0	7.9
1939	3.8	1.7	12.7	5.9	29.2	9.5	29.7	9.7	1.2	2.4	5.1	12.2	3.3	3.6	3.8	4.2	4.5	4.5	7.0	6.9
1938	5.0	2.3	16.3	7.5	24.9	8.1	24.6	8.1	2.7	6.5	5.1	12.2	4.3	4.7	6.0	6.5	2.4	2.4	12.0	11.9
1937	7.5	3.4	17.2	7.9	14.5	4.6	27.9	9.2	5.9	14.0	7.9	19.0	6.8	7.5	6.0	6.5	2.5	2.5	6.0	5.9
1936	7.7	3.5	20.6	9.5	9.4	3.1	34.3	11.1	4.1	9.7	5.7	13.8	10 2	11.1	13.0	14.2	7.3	7.3	8.5	8.4
1935	9.1	4.2	20.3	9.4	13.2	4.3	31.8	10 3	3.6	8 6	3.5	8 4	12.7	13.9	13.6	14.7	5.9	5.9	8.5	8.4
1934	29.0	13.3	28.9	12.9	29.6	9.5	30.8	10.0	5.2	12 6	4.1	9 9	7.6	8 3	9.2	10.2	12.6	12.4	13.0	12.9
1933	36.7	16.8	37.2	17.1	32.3	10.5	33.2	10.8	5.7	13.8	3.8	9.2	14.6	15.9	14.5	15.8	14.8	14.6	15.2	15.1
1932	52.1	23.9	51.5	23.6	33.0	10.9	31.7	10.3	5.7	13.8	3.8	9.2	21.4	23.4	22.3	24.4	23.5	23.0	22.7	22.6
1931					25.4	8.2	28.2	9.2												
Total	155.7	71.3	218.4	100.0	247.2	80.3	307.3	100 0	34.9	83.8	41.5	100.0	82.3	80.9	91.6	100.0	88.9	80.0	100.9	100.0

over, sections of branches in these areas reveal that approximately one-third of the xylem has broken down. Before 1934 the growth rates of the normal and damaged branches were practically identical. After the injury, however, the growth rate of the affected branches decreased progressively each year, until, at the end of the six-year period, their total growth was only about 71 per cent. of that of the normal branches (fig. 1).

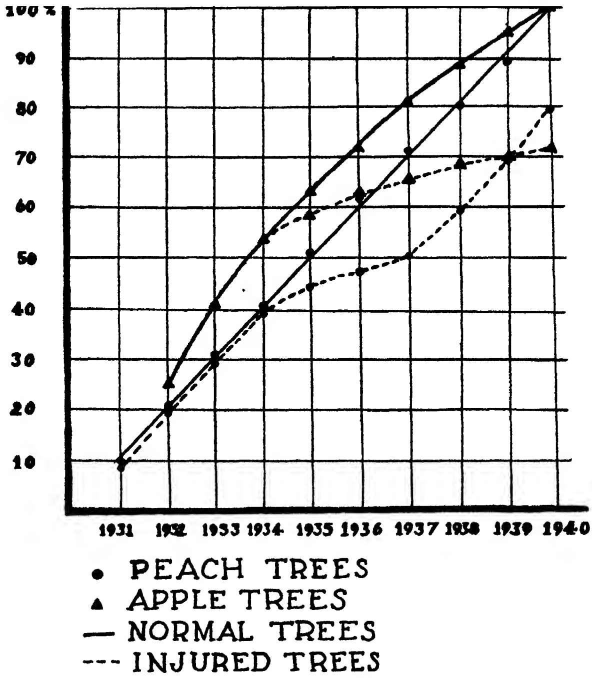

FIG. 1. Percentage of growth in various trees following cicada injury, 1931–1940.

Over the same period, the peach trees showed a decrease in total growth of about 20 per cent. (fig. 1). The decrease in growth rate was most marked in the three years immediately following the injury. After this time the growth of the damaged branches paralleled that of the normal branches. Sections through the injured areas revealed a breakdown of about 10 per cent. of the xylem in these areas.

The non-orchard varieties showed a variation of between 10 and 20 per cent. in the growth of the normal and injured branches (table I).

Discussion

There seems to be no doubt that the injuries inflicted upon the trees by the adult cicadas, in the course of their egg-laying activities, do affect the future growth of the branches. The decrease in the growth rate seems to be directly proportional to the breakdown of the water-vascular system.

In the case of the peach trees, the return to a normal growth rate seems to be attributable to several factors: 1) the extent of the injury; 2) the vitality of the trees; and 3) the care given the trees. As has been pointed out only about 10 per cent. of the xylem in these trees was affected. The trees, being quite young, were growing very rapidly at the time of injury, and the damage was quickly repaired. Consequently, the amount of conducting tissue, while insufficient for two or three years, was rapidly increased, so that at the end of this time it was sufficient to take care of the needs of the branches, and growth proceeded at a normal rate.

Then, as these trees were younger and less severely injured, greater efforts were made to save them. They were cultivated and pruned oftener than the apple trees, and this undoubtedly facilitated the absorption of a greater amount of moisture.

In the case of non-orchard varieties, the decrease in growth seems to be directly proportional to the extent of injury.

It may then be concluded that injuries to trees, caused by such insects as cicadas, while often too slight to result in the death of the affected branches, may influence their later growth. The extent to which the growth rate is modified is proportional to the degree of injury, as reflected in the amount of conducting tissue destroyed. The age and vitality of the tree at the time of injury, and the care given the tree after injury, are factors which tend to influence the later growth of the damaged tree.

Summary

1. The egg-laying activities of the cicada, *Magicicada septendecim,* cause injuries to trees that affect their later growth.

2. The extent to which the growth rate is affected is proportional to the degree of injury.

3. The effect is apparently the result of the destruction or modification of water-conducting tissue (xylem).

4. The age and vitality of the tree at the time of injury, and the care given the tree after injury, are factors which tend to modify the harm done to the tree.

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BRIEF PAPERS

A SIEVE TUBE TRANSLOCATION MODEL

RAYMOND E. GIRTON

(WITH ONE FIGURE)

The mechanism of solute transport in the phloem suggested by DE VRIES (2) and favored by CURTIS (1) stresses protoplasmic streaming as the agent responsible for rapid translocation. The effectiveness of much a system can be shown to students of plant physiology by means of the simple demonstration model herein described.

A piece of glass tubing, (b, in fig. 1) 2×45 cm., constitutes the enlarged "sieve tube." This tube is divided into two cells by means of a porous "sieve plate" (e, and e^1), which is constructed of a disk of wire gauze. The disk is held in position by means of two thin sections of rubber tubing. This tubing should be large enough to fit the inside of the glass tube snugly and thus prevent the disk from sliding out of position. A solid rubber stopper (a_2) is inserted into the lower end of the tube and a one-hole stopper (a_1) inserted into the upper end.

Prior to the actual operation, the upper stopper is removed temporarily, the tube is filled with water and clamped into a vertical position on a ring stand. Two 25-watt electric light bulbs (d_1 and d_2) are also clamped into position as shown in the diagram. The light bulbs are placed very close to the side of the tube and near the lower ends of the cells. When the lights are turned on, uneven heating of the water within the two cells results and convection currents (c) are set up. These currents constitute the "protoplasmic streaming" and continue indefinitely owing to the maintenance of temperature differences on the two sides of the glass tube.

The nature and rate of the "streaming movement" can be observed by adding very small particles of sawdust to the surface of the water in the upper cell. These particles should be fine enough to pass through the perforated disk with ease. A porous Gooch crucible has been found to serve as a satisfactory sieve for obtaining sawdust particles of the proper size. The smaller particles, particularly, remain in suspension for some time and trace the course of the streaming "protoplasm" up the illuminated side and down the far side of the cells.

In order to demonstrate the rapid longitudinal transport of solutes, an aqueous dye solution may be added to the upper cell and its progress followed as it is first moved about the upper cell and then across the "sieve plate" and into the lower cell. Solute movement in the reverse direction can be demonstrated by injecting dye into the bottom of the lower cell and

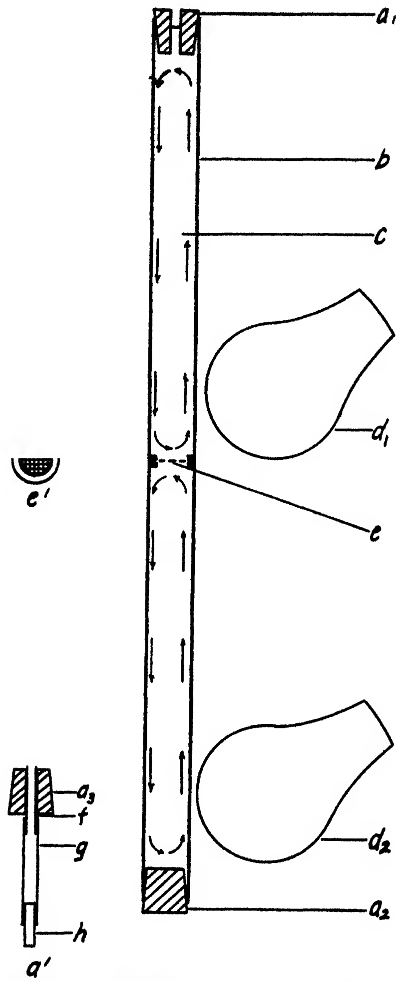

FIG. 1. Sieve tube model with details e′ and a′. See text for explanation of symbols.

watching its upward movement from cell to cell. Finally, the simultaneous movement of solutes in opposite directions can be demonstrated in the same sieve tube "element" by adding a few drops of a dye solution of one color such as methylene blue to the upper cell and, at the same time, injecting a second dye solution of a different color, safranin, into the lower cell.

In figure 1 the inset labelled a[1] shows a device which can be used for injecting dye solutions into the lower cell. The injector consists of a one-hole stopper (a_3), a short section of glass tubing (f), a piece of rubber tubing (g), and a glass plug (h). The device is charged with dye as follows: a clamp is applied near the upper end of the rubber tubing, the injector

inverted, and the rubber tubing filled with dye solution after momentarily removing the plug. The injector is now placed in an upright position and the section of glass tubing filled with water. In actual use, the device is inserted in the place of the solid stopper (a_2) in the lower cell. Injection of dye into this cell is effected by first releasing the clamp on the rubber tubing, and then applying other clamps to the tubing in such a way as to force the dye solution out of the injector and into the lower cell.

If it is desired to increase the complexity of the model, an additional cell, or cells, may be added. This necessitates the use of a longer tube and more "sieve plates." Each cell of such a model should be provided with its own light bulb in order to insure the necessary "streaming" activity.

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USE OF NITRIC ACID IN CONTROL OF pH AND NITRATE LEVELS IN NUTRIENT SOLUTION

FRANK M. EATON

TRELEASE and TRELEASE (2) have called attention to the advantages, from the standpoint of pH control, of using mixtures of ammonium and nitrate salts in making up nutrient solutions. Sulphuric acid is customarily employed as a means of adjusting the pH of solutions during an experiment. The purpose of this paper is to call attention to the advantages of using nitric acid as a means of maintaining at one and the same time both H-ion concentrations and nitrate levels. The writer has used nitric acid for this purpose over a number of years with considerable success.

TABLE I

VARIATIONS IN pH WITH THE ADDITION OF NITRIC ACID

DATE	pH*	ACID ADDED*	TRANSPIRATION	LIGHT INTENSITY†	EVAPORATION‡
		ml.	*liters*	*gm. cal./sq. cm./day*	*ml.*
Sept. 14	.	400	82	528	798
15	...	......	109	499	960
16	6.0	......	121	525	1090
17	6.3	50	133	503	908
18	6.2	50	89	399	832
19	6.1	......	70	307	765
20	6.2	50	82	280	1090
21	6.1	50	105	397	1108
22	5.9	......	93	364	973
23	6.1	50	67	269	984
24	5.9	......	rain	257	
25	5.5	......	......	72	430
26	5.7	......	33	304	334
27	6.1	50	43	441	472
28	5.9	......	43	435	453
29	5.9	......	45	456	502
30	6.1	50	47	415	506
Oct. 1	6.1	......	43	364	437
2	5.9	......	54	426	504
3	5.9	......	50	413	476
4	6.1	50	54	466	568
5	5.7	......	52	450	563
6	5.9	......	30	257	221

* pH readings and acid additions were made at 8: 00 A.M. and 9: 00 A.M. respectively.
† Eppley pyrheliometer, University of California, Citrus Experiment Station.
‡ Circular shallow pan evaporimeter, 0.1 sq. meter.

The extent to which nitrate levels can be maintained by periodically adjusting the pH to some selected level with nitric acid is contingent upon the kind of plant, its stage and condition of growth, and climatic factors (par-

ticularly light intensities) and the selected pH. The writer's work has been entirely in the Southwest where bright days and upward trends in pH are the rule. All of the cultures have been carried at a pH of about 6.0.

For the purpose of illustration, an example is taken from 1939 experiments wherein plants were grown in large out-of-doors sand cultures (1, fig. 4) in salt toxicity studies. These sand beds were supporting 30-inch rows each of milo, cotton, squash (harvested September 18), alfalfa, sugar beets, cowpeas, and tomatoes planted in mid July. The volume of the solution reservoirs was 2400 liters with an additional 300 liters retained in the sand, thus permitting the addition of readily measurable amounts of concentrated nitric acid. In this system, 50 ml. of nitric acid (sp. gr. 1.42) introduces 0.27 m.e. of NO_3 ion per liter. Using tap water to replace transpiration losses, new solutions starting with 7.5 to 8.0 m.e. per liter of NO_3 ion at pH 6 typically contain from 7 to 9 m.e. at the time the solutions are discarded. Similar effects have been observed in the parallel experiments conducted under cool coastal conditions near San Diego, under the desert conditions of

TABLE II

ANALYSES OF NEW AND OLD SOLUTIONS AND OF TAP WATER ADDED TO REPLACE TRANSPIRATION AND EVAPORATION LOSSES

SOLUTIONS ANALYZED	PERIOD SEPTEMBER 15 TO OCTOBER 7 (SEE TABLE I) MILLIEQUIVALENTS PER LITER							
	Ca	Mg	Na	K	HCO_3	SO_4	Cl	NO_3
New solution	4.90	3.80	1.96	1.66	0.38	4.28	1.72	7.42
Old solution	5.81	3.60	2.69	0.50	0.35	4.71	1.60	7.44
Tap water	1.84	0.56	1.60		2.90	0.61	0.60	0.05

the Coachella Valley, and under the intermediate climatic conditions represented at Riverside. The period represented in table I was taken from the Riverside data because it illustrates the increased H-ion concentrations of the solution during a period of low light intensity during which absorption of cations exceeded that of anions. Additions of phosphate and potassium are necessary during any extended use of a solution. Table 2 reports analyses of the new and used solution and of the tap water used in making up and replenishing solutions. Five m.e. of nitrate were added as calcium and potassium salts in making up this solution and then sufficient HNO_3 was added to bring the pH down to 6.0. Results comparable with the foregoing have been obtained in the beds receiving toxic concentrations of chloride and sulphate salts and also in small greenhouse sand cultures supporting single species of plants.

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NOTES

Eighteenth Annual Meeting.—The eighteenth annual meeting of the American Society of Plant Physiologists will be held at Dallas, Texas, on December 29–31, 1941. The headquarters of the Society will be the Baker Hotel. Plans for the meeting are well advanced, and it is hoped that the attendance will be large. The annual dinner for all plant physiologists has been arranged for the evening of December 29, probably at the Baker Hotel, and members should obtain tickets as soon as possible after arrival. The STEPHEN HALES address is usually a feature of this gathering, and the announcement of the CHARLES REID BARNES life membership award has been an annual occurrence at the dinner for 15 years. No one should miss these good-fellowship occasions. Two symposia are planned, one on cellular electro-dynamics, and the other, a joint meeting, on the application of statistical methods to physiological research. These are pleasing changes in subject matter, and the symposia will be extremely valuable to all who can attend them. Dallas has many attractions to offer the visitor, and meetings seldom go into this region. This is an opportunity to learn something about the great Texas empire, and to enjoy rare scientific fellowship.

Summer Meeting.—The New England Section of the American Society of Plant Physiologists was host to the Society at Durham, New Hampshire, on June 25 and 26, 1941. Usually the Section meets in May, but this year joined with the Society in participation in the 75th anniversary celebration of the founding of the University of New Hampshire. The A.A.A.S. held its annual summer meeting at Durham, and our meeting was a part of this general program. The officers of the New England Section did everything that could be done to make the meeting pleasant and profitable. Dr. T. G. PHILLIPS, chairman of the section and chairman of the local committee, and Dr. LINUS H. JONES, secretary of the section, deserve the thanks of everyone who attended for their skillful handling of the meeting.

"Auxins and their relation to growth in plants" was the subject of the round table discussion on the afternoon of June 25. Dr. FOLKE SKOOG opened with a discussion of the relation between extractable auxin and growth; Dr. SANDER covered the field of dwarfing as related to auxin; and Dr. THIMANN reviewed the mechanism of auxin action. Dr. G. S. AVERY, Dr. R. H. GOODWIN, and Dr. B. COMMONER contributed informal comments, and there was much general discussion of the many difficult angles. The presentations were excellent, but left one unsatisfied, possibly because of the more or less chaotic condition of the field. If the editor may be permitted an observation as an interested listener, it would be that it seems yet too early for an integration of the work that has been done. It will possibly

take some years of more careful research before we can judge the value of the innumerable contributions of recent years, many of which came out prematurely.

Thursday, June 26, was devoted to short papers during the morning session, and to a joint session with the American Society for Horticultural Science at the afternoon session. Many interesting papers were submitted for discussion.

The New England Section business meeting was held at 9:00 A.M. on June 26, and it was a pleasure to note the fine spirit and the apparent vitality of the organization. The officers selected for 1941–1942 are as follows: Chairman, Dr. DOROTHY DAY, Smith College; vice chairman, Dr. KENNETH V. THIMANN, Harvard University; secretary-treasurer, Dr. LINUS H. JONES, Massachusetts State College. In 1942 the annual meeting will be held in May, as usual.

Western Section.—The Pasadena meetings of the Western Section, held from June 17th to 21st, were very well attended (attendance ranging from 60 to 200). Three symposia were held jointly with the Botanical Society of America, the American Phytopathological Society, the Western Society of Soil Science and the American Society for Horticultural Science. D. R. HOAGLAND presided at the symposium on micronutrient deficiency diseases of crops. The characteristics of zinc deficiency were discussed by W. H. CHANDLER, who stressed the point that zinc deficiency causes more trouble in good soils than in poor soils and that woody plants are more susceptible than herbaceous plants. E. A. OVERHOLSER reviewed boron deficiency which occurs in the Northwest. Boron added to soils is not very effective because it is adsorbed in the upper layers. Deficiency diseases of vegetables in the east were reviewed by J. E. KNOTT, who showed that many organic soils improve greatly when copper, manganese, and iron are added. J. P. BENNETT stressed the importance of the availability of iron. Inorganic iron is available only in acid media; organic iron is rapidly destroyed by micro-organisms. Colloidal iron (humate) is available to the plant over a pH range from 3 to 9, probably due to contact absorption. Finally, W. T. MCGEORGE discussed some aspects of the chemistry of soil, stressing especially "acidulated fertilizers."

A symposium on plant hormones under chairmanship of F. W. WENT was given. The chairman discussed the chemical specificity of the auxins pointing out the minimum structural requirements for a chemical to cause cell elongation. J. P. BENNETT showed evidence of the presence of a substance in the buds and bark of trees and other plant material which breaks dormancy of apple and peach trees. JAMES BONNER pointed out that the popular notion that vitamin B_1 will promote the growth of horticultural

plants is no longer tenable. J. VAN OVERBEEK discussed dwarfism in corn, which is due to excessive auxin destruction. He furthermore showed that, by injecting naphthaleneacetic acid into ovaries of Datura, a fruit develops with well developed "seeds" which contain an as yet non-viable pseudo-embryo.

A symposium on protoplasm was given, headed by O. L. SPONSLER who discussed the relative size of the various constituents of protoplasm. A. R. MOORE discussed such aspects as the emulsion theory of structure and surface tension, and minimized its importance. A. L. COHEN, however, attached greater importance to surface tension as a cause of movement and also as a responsible agent in its determination. A very instructive motion picture showing division of chromosomes, nuclei, and of cells growing *in vitro* concluded the symposium.

Twenty-eight miscellaneous submitted papers were presented. The functioning of completely air-conditioned greenhouses at the California Institute of Technology was discussed by F. W. WENT, who also presented another paper showing how a constant temperature of 26.5° C. gives good vegetative development but prevents fruit setting in tomatoes; for the latter, cooler nights are essential. F. T. ADDICOTT showed that reduced meristematic activity is the main characteristic of isolated tomato roots growing in media lacking vitamin B_6. H. E. HAYWARD discussed the occurrence of a highly suberized layer in the root cap of orange seedlings going into dormancy due to high chloride concentrations. M. A. JOSLYN discussed the formation of succinic acid by yeast cells. P. W. ROHRBAUGH measured biologically the amount of ethylene in motor exhaust gases. G. H. HARRIS stressed the importance of sulphur for the growth of raspberries. A highly instructive demonstration was given by W. Z. HASSID, who showed that glucose phosphate (Cori ester) is immediately converted into synthetic starch when phosphorylase prepared from potato juice was added. A. GOETZ discussed the extreme susceptibility of yeast cells to silver ions. D. R. HOAGLAND and T. C. BROYER discussed experiments showing the importance of respiration for accumulation and permeability of roots. Permeability to bromine was decreased if nitrogen instead of air was bubbled through the solution around the roots. Cyanide prevents accumulation. Guttation liquid of barley plants is about half as concentrated as xylem exudate of decapitated plants. Plants having their roots in distilled water do not guttate. J. VAN OVERBEEK showed evidence of water uptake by forces other than osmosis by comparing the osmotic pressure of the exudate with the osmotic pressure of mannitol solutions which just prevented bleeding. R. EMERSON showed that the phycocyanin pigment of blue-green algae is effective in photosynthesis. It may, however, only absorb energy and then transfer it to chlorophyll *a*. P. J. ALLEN demonstrated a 700 per cent. increase

in respiration of wheat leaves as a result of infection with powdery mildew. D. M. BONNER discussed 4 groups of chemicals (amino acids, purines, organic acids, and inorganic salts), which promote growth of isolated sections of leaf tissue. Adenine is effective in the lowest concentration and may be regarded as a leaf growth hormone. JAMES BONNER showed that vitamin B_1 is translocated in the phloem in a similar manner to other plastic materials. It accumulates above a girdle, in contrast to vitamin B_2 which apparently did not show this effect. D. I. ARNON showed that plants at pH 3 do not grow because they are unable to absorb nutrients. At pH 9 all elements are taken up although at a reduced rate, with the exception of phosphorus, thus explaining why plants grown at high pH show symptoms of phosphorus deficiency. The pH of expressed sap remained constant throughout the pH range. G. F. LEIBIG showed that the presence of small amounts of aluminum increases the tolerance for copper. W. W. ALDRICH reported a starch content of 55 per cent. in trunks of date trees prior to fruiting which rapidly decreases when the fruit ripens. The impossibility of maintaining soil moistures below field capacity was stressed by A. H. HENDRICKSON. S. H. CAMERON showed that heavily pruned trees regenerated less top growth and have a smaller yield than lightly pruned trees. The yield of lightly pruned trees was below that of check trees. Two papers on carotenes were presented; one by O. F. CURTIS, who showed that the carotene content decreases in mineral deficient plants with the exception of plants low in phosphorus. A. L. LEROSEN showed that in tomato fruits the development of red and yellow carotenoid pigments depends on the presence of a gene R. The skin contains less of these pigments than the flesh. A. S. CRAFTS discussed a paper by R. N. RAYNOR on selective toxicity of certain phenol compounds on different plants. The effect depends on the relative permeability of the cuticle to fat and water soluble substances. W. O. WILLIAMS demonstrated improvement in the colorimetric determinations of potassium with dipicrylamine.

Excursions were held to the laboratories at Riverside and to the Santa Ana Botanic Gardens. Officers elected for the coming year: Chairman, J. VAN OVERBEEK; vice chairman, E. J. BARTHOLOMEW; secretary, D. I. ARNON.

Barnes Committee.—President EDWIN C. MILLER of the American Society of Plant Physiologists has named the following committee to choose the recipient of the eighteenth CHARLES REID BARNES life membership award:

Dr. PAUL WEATHERWAX, Indiana University;
Dr. EDITH ROBERTS, Vassar College;
Dr. W. M. ATWOOD, Oregon State College;
Dr. PAUL R. BURKHOLDER, Yale University;
Dr. W. E. LOOMIS, Iowa State College, chairman.

This award, established in 1925, has been made annually since 1926. The committee has an unusual opportunity and privilege in making the selection. There are 14 living members at the present time who have been honored in this manner.

Changes in Constitution.—All of the changes in the Constitution of A.S.P.P. voted upon in the last election were adopted. The new version of this instrument was published in Bulletin no. 13, and is in the hands of all members. On rereading and mature deliberation, the editor believes that one of these changes represents a serious backward step. He will be glad to explain the serious difficulty which may arise if article VIII, section 2 is permitted to remain in its present form. The American Society of Plant Physiologists has rightly prided itself on its rather rigidly democratic nominations and elections, which could not be manipulated by small groups acting in concert. The present version has lessened the rigorous restraints, and we have had a clear demonstration already of the great value of one feature of the original provisions, under which the elections of 1941 were held. We trust that many members will interest themselves in this matter, and that section 2 of article VIII may again receive keen attention from the membership in general. No society is any better than its nomination and election machinery, and we cannot afford anything but the best.

Dedication.—It is a pleasure to carry out the expressed wishes of the American Society of Plant Physiologists to dedicate this number of PLANT PHYSIOLOGY to Dr. ALEXANDER PIERCE ANDERSON, and to present the fine portrait of him as the frontispiece of the October number. Dr. ANDERSON will be seventy-nine years old on November 22, and on behalf of all members of the Society, we extend to him and his family, sincere congratulations on that happy occasion, and our good wishes for the coming years.

Conservation of Space.—In view of the uncertain conditions abroad, and the already heavy inroads that war has made on our foreign memberships and subscriptions, it is necessary to exercise the utmost caution in use of the Society's funds. Every time the number of offices increases, every time we undertake any new projects, heavier expenses are entailed before any publishing can be done. We urge all writers to condense their papers, to use cuts sparsely, and as few tables as possible. Papers should be short, clear, and deal mainly with new information. Long introductions, literature reviews, and elaborate details of methods already described in other papers should be sheared as much as possible. Please conserve space and expense in preparation of papers intended for PLANT PHYSIOLOGY. The cooperation of all authors is solicited.

Errata.—As in previous volumes, an errata list follows the table of contents of volume 16. Thanks are extended to the authors who have aided in making these corrections. Some of them could be avoided if authors would use more care in proofreading their galleys, especially the tables, where errors are hard to detect in editorial proofreading. Enter the corrections in your copies; it is a good habit.

Necrology.—Death has recently removed from among us two well known members of the American Society of Plant Physiologists. In accordance with our usual custom, brief notices are presented.

WILLIAM FRANCIS GANONG

Dr. WILLIAM FRANCIS GANONG died at St. John, New Brunswick on September 7, 1941, after a long illness. He is survived by his widow, and two children. A very brief account of his life appeared in the January, 1941 issue of PLANT PHYSIOLOGY, in connection with the award to him of the CHARLES REID BARNES life membership at the Philadelphia meeting. A more complete biography has just appeared in Science for October 3, 1941, prepared by his associates, Dr. FRANCES GRACE SMITH, and Dr. HELEN A. CHOATE, of Smith College. Dr. GANONG's influence will live long in the annals of plant physiology, to which he had devoted a lifetime of interest. Everyone who knew him and his work will learn of his death with profound regret.

WALTER JOSEPH HIMMEL

In the death of WALTER JOSEPH HIMMEL, of the University of Nebraska, the Society has lost one of its early members, as his membership dated from the establishment of an official journal. At that time he was associate professor of biology at Macalester College, St. Paul, Minnesota. Since 1927 he has been with the Department of Botany at Nebraska, and several of his papers have been published in PLANT PHYSIOLOGY. His scientific interests were broad and varied, and he held memberships in societies devoted to ecology, genetics, general botany, and ornithology. One of his hobbies was music, and he was an accomplished director, and player of the trumpet. His higher degrees were obtained at Iowa, M.S., 1922; and Ph.D., 1924.

He was born at Radcliffe, Iowa, on April 20, 1889, and died on July 23, 1941, while visiting at Iowa Falls, Iowa. He is survived by Mrs. HIMMEL and their son, to whom we express our keen sympathy.

Annual Review of Biochemistry.—The tenth volume of the Annual Review of Biochemistry was recived in June, too late to be noticed in the July number of PLANT PHYSIOLOGY. There are 24 reviews, and in spite of

the difficulties imposed by world conditions which prevents receipt of journals and reprints from abroad, they give a clear picture of the advances made in the fields covered. The reviews included in this number are as follows: Biological oxidations and reductions, by E. S. Guzman Barron; proteolytic enzymes, by M. Bergmann and J. S. Fruton; nonproteolytic enzymes, by H. Tauber; chemistry of the carbohydrates and glycosides, by A. G. Norman; chemistry of amino acids and proteins, by M. S. Dunn; the chemistry and metabolism of the compounds of sulphur, by A. White; carbohydrate metabolism, by C. F. Cori and G. T. Cori; fat metabolism, by H. Eckstein; the metabolism of proteins and amino acids, by R. Schoenheimer and S. Ratner; the biochemistry of the nucleic acids, purines, and pyrimidines, by F. W. Allen; the biochemistry of creatine and creatinine, by H. H. Beard; detoxication mechanisms, by J. A. Stekol; hormones, by E. C. Kendall; the water-soluble vitamins, by H. A. Mattill; nutrition, by H. K. Stiebling and R. M. Leverton; relation of soil and plant deficiencies and of toxic constituents in soils to animal nutrition, by L. A. Maynard; mineral nutrition of plants, by A. L. Sommer; plant growth substances, by W. J. Robbins and V. Kavanagh; spectrometric studies in relation to biology, by T. R. Hogness and V. R. Potter; review of bioluminescence, by E. N. Harvey; the chemistry and metabolism of bacteria, by H. A. Barker; biochemical nitrogen fixation, by D. Burk and R. H. Burris; and properties of protein monolayers, by E. Gorter.

A short biography of Carl Lucas Alsberg, formerly a member of the editorial committee of this annual, whose death occurred late in 1940, precedes the reviews. The names appearing on the editorial committee for the first time, are H. J. Almquist and H. A. Spoehr.

The service being rendered to chemical biology by the *Annual Review of Biochemistry* is of incalculable value. Every student and research man in this field should have access to the complete file, and make use of it as a guide to the trends and significance of biochemical progress.

The work is obtainable from Annual Reviews, Inc., Stanford University, at $5.00 per copy.

GENERAL INDEX[1]

[1] Papers published in PLANT PHYSIOLOGY are indexed in *The Agricultural Index*, The W. H. WILSON Co., New York.